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THE JOURNAL OF PHYSIOLOGY

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BY

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THE INFLUENCE OF DIETS LOW IN MAGNESIUM
UPON THE CHEMICAL COMPOSITION OF
THE INCISOR TOOTH OF THE RAT

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ALTHOUGH Mg is quantitatively one of the most important of the minor constituents of the inorganic material of teeth, little is known of the effects of dietary deficiency of the element on tooth formation. Watchorn & McCance [1937] studied conditions arising in subacute Mg deficiency, but as their experimental animals survived for 12 weeks their findings are not applicable to cases of acute Mg deficiency. We have been unable to find any record of chemical studies of the teeth in acute Mg deficiency, or any record, either chemical or histological, of the effects of restitution of Mg in the diet after periods of deficiency. The present communication attempts to remedy this hiatus to some extent and to offer an explanation of the observed histological and chemical changes.

METHODS

Two experiments were conducted. In the first, the lower incisors were used for chemical examination and the upper incisors of the same rats for histological examination [Irving, 1940]. In the second experiment, both upper and lower incisors from eight rats in each group were used for the chemical and the upper incisors from two rats, litter-mates of the former, for histological examination. The following procedure was adopted throughout.

Management of animals

The rats were weaned at 25 days of age and were kept in galvanized iron cages housed in an almost sound-proof, constant temperature (25° C.) room and were offered the diets and glass-distilled water *ad lib.* The use of an almost sound-proof room was essential in the present investigation since auditory stimuli precipitate attacks of tetany in Mg-deficient rats.

Apparently spontaneous attacks occur from the sixth day onwards, even under the most strictly controlled conditions, and many of the subjects die in the first seizure. For this reason, in the curative experiments recorded here, the deficiency periods were limited to 6 days from weaning, the subjects being then either killed or transferred to the adequate regimen.

Diet

A Mg-deficient diet containing not more than 0.0006% Mg (i.e. 6 p.p.m.) was prepared. Full details of the methods of purification of the different constituents of the diet and the mode of preparation are recorded elsewhere [Duckworth, Godden & Warnock, 1940]. The adequate diet was prepared by adding sufficient MgSO_4 to the deficient diet to raise the Mg content to 0.07%. Rats fed the adequate diet showed an average growth curve identical with that of litter-mates receiving the stock diet of the main colony, supplemented with greens and skimmed milk.

Analyses

Mg was estimated by the method of Godden & Duckworth [1935] after removal of Ca by the method of Clark & Collip [1925]. Samples were ashed and the ash was dissolved in hot dilute HCl and adjusted to a known volume when cold.

RESULTS

Exp. 1. Pairs of rats were killed after different periods on the diets. In some cases, however, rats on the deficient diet with relatively long survival periods died in tetanic seizures and the teeth from them were then used. The lower incisors of pairs of rats were bulked and analysed for Mg. The results are shown in Fig. 1 *C*.

It will be noted from Fig. 1 *C* that the duration of the deficiency had no effect upon the total weight of Mg in the teeth, and such changes as did occur represented normal experimental variation. Tooth weight increased regularly throughout the experiment at probably a slightly subnormal rate. During the same period, the rats on the adequate diet showed an increase in total tooth Mg. In the restitution experiment, begun after 6 days on the deficient ration, it is clear that Mg was being deposited in the teeth at an approximately normal rate.

The constancy in the total weight of tooth Mg in animals on a deficient diet is in contrast to the fall in weight of bone Mg which occurs under similar conditions. For comparison with the data from the teeth results obtained from leg bone analyses on rats under conditions of deficiency, adequacy and restitution are given in Fig. 1 *B*. Those rats

receiving an adequate diet showed a steady increase in the amount of femur Mg, while those on the deficient diet showed a marked reduction. Restitution of Mg in the diet after a 6-day period of deficiency resulted in a rate of increase in femur Mg which was greater than that in the rats receiving an adequate diet throughout. This accelerated deposition and

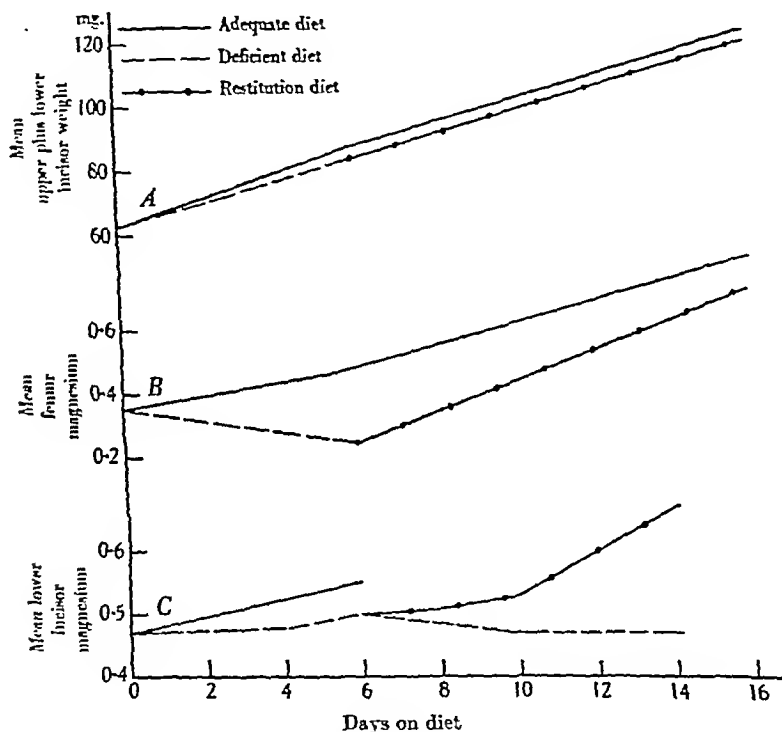


Fig. 1. Chemical results. A. Weight of dry fat-free upper and lower incisors of rats on different diets. Data from Exp. 2. B. Weight of Mg in the left femur of rats on different diets. Data from Exp. 2. C. Weight of Mg in the lower incisors of rats on different diets. Data from Exp. 1.

the earlier reduction in femur Mg during deficiency are indicative of the mobilization and the subsequent replenishment of a reserve of Mg. (These bone data are given elsewhere in full and with collateral studies on the whole skeleton [Duckworth *et al.* 1940].)

Exp. 2. Seven rats from each of eight litters were chosen and divided into five groups so that each group contained five male and five female rats, the extra rats being allocated to groups 3, 4 and 5. Eight rats from

each group were used for chemical studies and the remainder for histological examination [Irving, 1940]. The experimental treatment of the respective groups was as follows:

Group 1. Killed at weaning.

Group 2. Killed after 6 days on the deficient diet.

Group 3. Killed after 6 days on the adequate diet.

Group 4. Fed the deficient diet for 6 days and then transferred to the adequate diet for 10 days after which they were killed.

Group 5. Killed after 16 days on the adequate diet.

The results are given in Fig. 1A.

Although it was originally intended to separate the dentine and enamel by the flotation technique of Brekus & Armstrong [1935], it was found that the technique described for the separation of dentine and enamel of human teeth did not yield good results with the rat. Microscopic examination showed that, even after prolonged pulverization, fragments of dentine remained attached to particles of enamel. The composite specific gravity of such heterogeneous particles was such that they floated at the surface of the bromoform-alcohol solution used for separation, while only the part of the enamel which was largely free from attached dentine sank to the bottom of the liquid. It seems probable that the difference in behaviour of human and rat teeth treated in the same manner arises from the large ratio of enamel to the area of the dentine-enamel junction in the human tooth as compared with the rat tooth. It is regretted that present circumstances prevent this study from being repeated using a different technique.

The curves shown suggest that tooth growth was retarded slightly during the period of deficiency. The slight difference was still evident after a 10-day period of restitution. It might be mentioned that other studies on the growth of the femora in these rats showed that there was a similar difference between adequate and deficient rats at the end of 6 days. Similarly, the difference remained and was perhaps somewhat accentuated after 10 days on the restitution diet.

Outward tooth growth

Since the rat's incisor increases in weight by the deposition of new calcified material together with outward growth, it is possible that the increases in weight noted in the previous section might have been due only to new dentine deposited.

In order to estimate the influence of Mg deficiency upon the rate of outward growth of the rodent incisor tooth, twelve rats were placed on

the Mg-deficient diet at weaning. Before being placed on the diet, the upper incisor teeth of six of these were scratched with a jeweller's screw head file on the labial side at the gingival margin. After 6 days on the diet, these six rats were killed, a file mark again made at the gingival margin and the teeth dissected out. The other six rats, after 6 days on the deficient diet, were returned to the adequate diet. After 1 day on this diet, a similar scratch mark was made on their upper incisors and they were kept for a further 6 days on the diet. They were then killed, the teeth again marked and dissected out. As mentioned elsewhere [Duckworth *et al.* 1940], fatal seizures occur in Mg-deficient rats from the sixth day onwards and therefore it was felt advisable to allow the animals 1 day on the adequate diet, after the deficiency period, before filing the teeth. Greenberg & Tufts [1938] found that injection of Mg did not prevent reaction to stimuli up to 4-5 hr. after Mg dosage in hyper-irritable animals on a Mg-deficient diet, but it is interesting to note that, in our experiments, in no case did convulsions occur, even after such stimulus as tooth filing.

The distance between the two marks was measured under the microscope, using a micrometer eyepiece and a 2 in. objective. On the low Mg diet, the average growth rate, computed for 1 week, was 2.7 mm. In the animals replaced on the adequate diet, the average rate was 2.9 mm. per week. The low Mg diet thus had in 6 days slowed to some extent the rate of growth of the incisors. The figures must, however, not be stressed as the accuracy of the measurement is not of a high order. Using six rats of the same age on stock diet, the average growth rate was found to be 2.9 mm. per week. This figure is slightly higher than that of 2.2 mm. given by Addison & Appleton [1915], but Fredericia & Gudjónsson [1936] have pointed out that the incisal growth rate of very young rats is higher than that of more mature animals. The agreement between our figures for normal rats and those from rats on the high Mg diet shows that the latter was adequate for tooth growth. These results confirm in full those reported above for changes in tooth weight during Mg deficiency and restitution, and show that outward tooth growth was affected in the same way as the weight increase.

Attempts were made to measure the growth rate of the lower incisors, but, owing to the oblique gingival margin, it was not possible to get accurate results.

DISCUSSION

The chemical findings recorded above are complementary to the histological findings of Irving [1940] in showing the changes in the tooth during Mg lack. The subnormal deposition of Mg during deficiency was parallel to the tooth's vain attempt to maintain normal architecture; while the laying down of normal dentine after restitution of Mg in the diet and the resumption of normal increase in Mg content were likewise simultaneous.

Mg deficiency slightly reduced the rate of tooth growth. During this period the Mg content of the tooth remained practically unchanged, instead of gradually increasing as in rats receiving a diet adequate in Mg. The combination of a constant level of total tooth Mg and increasing tooth weight resulted in a gradually diminishing percentage of tooth Mg. These results, however, can only be interpreted in terms of the abrasion which occurs in the case of the incisor teeth. Since the distal ends of the incisors are constantly wearing away, the fact that total tooth Mg remained constant shows that Mg was being deposited in the tooth at approximately the same rate as it was being lost by abrasion. This being so, the teeth must be regarded as totally distinct from the skeleton, which serves as a reservoir of mobilizable Mg, as shown by the data for the femora.

That Mg is not mobilized from the teeth in the rat under the severe conditions of acute deficiency is parallel with the findings of other workers in the field of Ca metabolism. Schour [1938], reviewing the literature on the effects of hyperparathyroidism, injections of parathyroid hormone and repeated gestations and lactations on tooth Ca, concluded that the tooth does not act as a reservoir of mobilizable Ca. Also Gaunt & Irving [1939] have shown that Ca and P lack affected the teeth much less than the skeleton. Further, preliminary experiments by Gaunt, Griffith & Irving [1939] showed that after a period of P depletion, injected radioactive P was proportionately taken up more by the skeleton than the teeth, indicating that the greater effect of the depletion had been upon the skeleton.

It can be concluded, therefore, that the role of Mg in the growth of the teeth is sufficiently important to necessitate its deposition even under conditions of acute deficiency, although the rate of deposition is less than normal. In common with other inorganic constituents of the tooth so far studied, Mg cannot be withdrawn during periods of Mg lack. It is significant to note that the Mg and Ca metabolism of the tooth differ markedly from that of bone.

SUMMARY

1. The growth of the incisor tooth of the rat is only slightly diminished by conditions of Mg deficiency which interfere greatly with body growth.
2. The absolute amount of Mg in the tooth is unchanged in acute Mg deficiency, when that of the skeleton falls greatly. This indicates that the Mg of teeth, unlike that of bone, during such deficiency not only cannot be mobilized but is actually deposited within the experimental period employed.

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THE INFLUENCE OF DIETS LOW IN MAGNESIUM
UPON THE HISTOLOGICAL APPEARANCE OF
THE INCISOR TOOTH OF THE RAT

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ALTHOUGH it is known that the histological appearance of the tooth is altered in magnesium deficiency, little work has been done on this subject. Some histological studies of incisors and molars in rats under conditions of acute Mg deficiency have been described by Klein, Orent & McCollum [1935]. The chief changes found in the teeth were the appearance of striations in the dentine and proliferation of the parodontal tissues. The striations in the dentine were presumably due to an intermittent interference with the normal calcification process, and Klein *et al.* suggested that they were related to the convulsive attacks which are typical occurrences in acute Mg deficiency. According to Tufts & Greenberg [1938], however, the diet used was deficient not only in Mg but also in vitamin B₂ complex. Watchorn & McCance [1937], studying conditions arising in subacute Mg deficiency, confirmed the findings of Klein *et al.*, but showed that the argument presented by these workers to explain the observed dentinal changes was untenable, since striations were found in subacute deficiency when convulsions did not occur. They did not, however, present any alternative interpretation. Becks & Furuta [1939] found degeneration of the enamel organ in acute Mg deficiency, but, as will be shown subsequently, the changes they observed are probably not specific for this condition.

Advantage was taken of the concurrent lines of work at this Institute upon Mg metabolism and upon tooth formation, to observe more fully the dental changes which occur during dietary Mg deficiency, and the recovery process after return to a diet of normal Mg content. The plan of the experiment was very similar to that described in the preceding paper [Duckworth & Godden, 1940]. The diet and management of the animals was the same as that used by these authors.

METHODS

Thirty-nine animals were placed on the deficient diet at weaning and four or more were killed and examined after 2, 4 and 6 days. A number of the remaining rats were then transferred on to the adequate diet and killed and examined after 2, 4, 6, 8 or 10 days. The remaining animals on the low Mg diet were allowed to go on till they died, the longest survival period being 23 days. Four control rats on the adequate diet from weaning were killed after 6 days and two further ones after 16 days on the diet. An adult female animal, about 1 year old, was placed on the low Mg diet and, after subsisting on it for several months, was killed and its teeth examined.

Longitudinal sections made of the upper incisor teeth were cut at 12μ and were stained with haematoxylin and eosin. The sections were examined with a micrometer eyepiece and a series of semi-quantitative measurements made. Only the labial side of the tooth was examined, but it was noted that the lingual side in general showed similar changes.

RESULTS

Animals on the deficient diet

No change was seen until the animals had been on the Mg-deficient diet for at least 4 days.

Dentine. After 4 days on the diet, the first characteristic change of Mg deficiency appeared. This was a sudden increase in the predentine width at a point about 3 mm. from the basal part of the tooth. This point will be referred to as the "predentine step" (Fig. 2). Beyond the predentine step the predentine width became roughly double. In one animal on the deficient diet for 4 days, the predentine was 15μ wide up to the step and 31μ wide beyond. After longer periods on the deficient diet, two steps appeared (Fig. 3), the width of the predentine being about three times the normal value beyond the second step. The distance from the base of the tooth to the first step was remarkably constant in all teeth, being about 3 mm. The predentine-dentine junction was very sharp throughout the whole tooth and showed none of the irregularity characteristic of Ca deficiency. The dentine formed during the deficiency did not, however, appear entirely normal. It had a translucent appearance, did not stain so deeply with haematoxylin, and the dentine tubules appeared unduly prominent. After 8 days on the deficient diet, the wide predentine showed the stratification of the predentine to which other authors have drawn attention. Careful examination of the teeth showed

this to be an abortive attempt at a homogeneous calcification of the, by now, very wide predentine, each stratification corresponding to a step in the more proximal part of the tooth. Thus if the predentine was followed from the proximal to the distal part, it was found to become suddenly wider beyond the step. At a variable distance distally, a line, staining deeply with haematoxylin, appeared in the predentine at a distance from the odontoblastic margin corresponding to the predentine width before the step. This line might continue to the tip of the tooth, or might disappear completely, or might reappear at intervals as the predentine was followed distally (Fig. 4). On the odontoblastic side the line was very sharp and straight, on the dentine side it merged gradually into the predentine. If two steps were present in the predentine, two lines were often found in the more distal part (Fig. 5). In no case, however, did fusion with the dentine occur.

In animals which had been on the deficient diet for some time, no definite predentine steps could be made out as they were masked by the stratification in the predentine. In the adult rat the dentine at the basal end of the tooth had a folded appearance very similar to that reported by Schour & van Dyke [1932] as occurring after hypophysectomy (Fig. 6; compare with Fig. 7). Prolonged vitamin A deficiency also causes similar changes [Wolbach & Howe, 1933].

The odontoblasts showed a progressive atrophy when the animals were on the deficient diet. The normal height of these cells is about 51μ ; after 4 days on the diet this was reduced to 40μ and after 23 days to 33μ . After 8 days on the deficient diet the odontoblasts in many places had not receded after laying down predentine and were embedded in this tissue, giving the line of odontoblasts a puckered appearance (Fig. 8). In two cases the odontoblasts had become calcified *in situ*, the pulp cavity appearing to be lined in places with osseous material (Fig. 9).

Enamel. After 6 days on the diet, the enamel organ showed large calcareous granules embedded in its substance at the basal end (Fig. 8). As the deficiency progressed these granules became more frequent, causing marked distortion of the enamel organ (Fig. 9). After 23 days on the diet the entire enamel organ showed atrophic changes. In the proximal part the four normal layers could no longer be distinguished and only ameloblasts and epithelial papillae remained. This part of the enamel organ indeed differed little in structure from the intermediate zone, and, in both, the epithelium and papillae had regressed in size. In the distal part the ameloblasts were replaced by a low cubical epithelium, and the shrunken epithelial papillae were seen on a background of

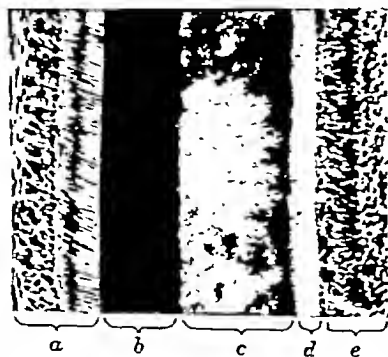


Fig. 1.

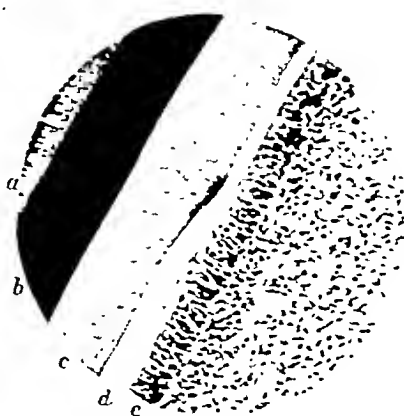


Fig. 2.



Fig. 3



Fig. 4.

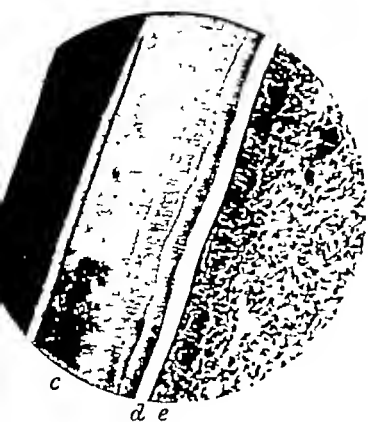


Fig. 5.



Fig. 7.



Fig. 6.



Fig. 8.

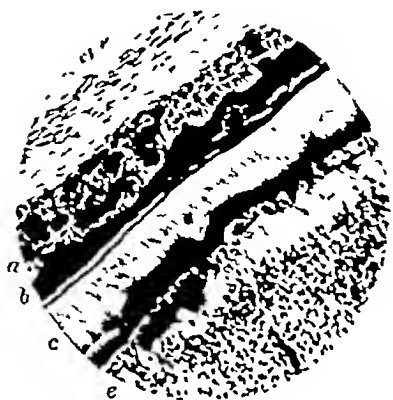


Fig 9

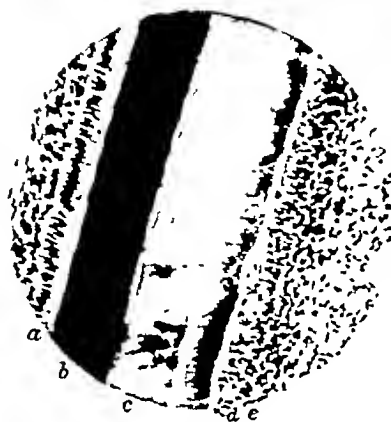


Fig. 10

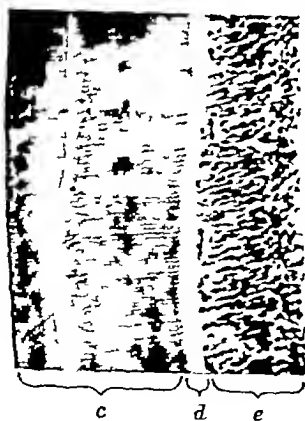
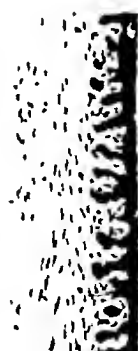


Fig. 11.



A



B



C

Fig 12

formed was less than normal until the animal had been on the adequate diet for about 6 days.

The predentine step moved slowly distally and was finally lost at the end of the tooth. After 4 days on the normal diet, the distance from the base of the tooth to the step was 4.6 mm. and after 8 days it had become 6.0 mm.

The odontoblasts rapidly recovered and regained their normal height, the new ones laid down at the basal end of the tooth being normal in all respects. Many of the more distal odontoblasts, however, had not recovered from the previous deficiency even after 10 days on the diet; these had been formed while the animal was still on the deficient diet and in places were deeply embedded in the new dentine.

Enamel organ. No new calcified granules were formed in the enamel organ, and those which were present and which had been formed during the deficiency period moved slowly distally the longer the animal was on the adequate diet.

DISCUSSION

The histological findings show that Mg deficiency exerted a marked influence upon tooth calcification. In the early stages, this process was retarded in a characteristic way; later, while still on the deficient diet, attempts to return to the normal calcification cycle led to stratification in the predentine, and adventitious calcification of the enamel organ and of the odontoblasts.

In the early stages of the deficiency, the extreme proximal end of the tooth remained normal, the predentine step being formed 2-3 mm. from the epithelial sheath. This abrupt alteration in predential width was the most specific change found and has not been described as occurring in any other condition. On it hung all the other changes in the dentine. There is no doubt that this and subsequent changes in the predentine were intimately related to the progressive atrophy of the odontoblasts, already described by Watchorn & McCance [1937], which was first noted after 4 days on the deficient diet, the stage at which the predentine step was also first seen.

Why the predentine step should occur at a relatively constant distance from the base of the tooth cannot be explained. It is, however, well known that the vascularity of the pulp becomes poorer in the more distal parts, and that changes in the predentine are always more extreme distally. It may possibly be that, at the level of the step, the vascular supply began to be insufficient to provide enough Mg when the intake was already inadequate.

Beyond the step, the calcification cycle was upset in a quantitative way. In all the deficient teeth examined, the predentine width had the normal value of about 16μ up to the step. Beyond this, the predentine became virtually twice as wide. This indicated a change in rhythm from a 24 to a 48 hr. cycle. When two steps were present, the cycle took 72 hr. to complete. That this is the true explanation, and not that the teeth had ceased to calcify altogether, which seems at first more obvious, was shown by the fact that at 6 days, when the wide predentine was very marked, the teeth were still growing almost normally. This had further been considered in relation to another point. The chemical findings showed that while the Ca and P content of the teeth, reckoned in absolute amount, continued to increase, the Mg content remained constant. It was thus possible that what appeared histologically as predentine was actually abnormally calcified dentine, low in Mg. But Schour & Ham [1934] have conclusively shown that normal dentine stains with haematoxylin and uncalcified predentine with eosin; and it appears improbable that this wide predentine was in fact calcified, since later in the deficiency calcified matter staining normally with haematoxylin was found not only in the enamel organ and odontoblasts, but in the predentine itself, and this calcified matter presumably likewise contained little Mg. I therefore consider that the original interpretation is the correct one, that the 1-day calcification cycle required 2 and later 3 days for completion. The diminution in inorganic matter, expressed on the increased width of the predentine, was probably far too small to contribute to the slight loss of tooth weight observed, the more so, since comparison of the growth and weight figures of the tooth show them to be in almost the same proportions. The slight change in appearance noted in the dentine during the deficiency may well have been due to its low content of Mg.

The later changes in the tooth, while the animal was still on the deficient diet, appeared to be an attempt to return to normal calcification. The effort to resume the 16μ cycle in the predentine failed as the incremental stratifications did not fuse with each other or with the dentine. Calcification was so extreme that the odontoblasts became calcified *in situ* or became included in the predentine.

Along with the abnormal calcification of the odontoblasts, adventitious calcareous granules were found in the more proximal parts of the enamel organ at and after 6 days on the deficient diet. This represented the earliest stages in the atrophy of the enamel organ which was so conspicuous after 23 days' deficiency and in the adult animal. This change has also been described by Becks & Furuta [1939] in Mg deficiency. I do

not, however, consider this as a change specific to Mg deficiency. Schour & van Dyke [1932] found very similar changes after hypophysectomy, and Wolbach & Howe [1933] have reported changes in the enamel organ in vitamin A deficiency which the writer has found to be indistinguishable from those caused by Mg lack (Fig. 12). It is not altogether surprising that several apparently unrelated factors should affect a highly developed structure like the enamel organ, which is not only laying down a very specialized tissue but which also exhibits continual growth.

As the teeth sections were decalcified it is impossible to say if the enamel removed by this process was in any way changed. The organic enamel appeared normal in structure save where it was distorted by the calcification in the enamel organ. In late stages of the deficiency, however, the amount of organic enamel was definitely reduced, indicating a much earlier calcification of the enamel.

It has been found by a number of workers [see review, Duckworth, 1938-9] that one of the outstanding characteristics of Mg deficiency is its effect upon Ca metabolism, prolonged deficiency causing adventitious deposition of Ca salts in the body. In the present investigation, a prominent change found in the teeth was the presence of calcified material in the enamel organ and odontoblasts, and, as just stated, in later stages the enamel appeared to be calcified earlier than usual. These changes were seen after only 6 days on the deficient diet; it would thus appear that, as was found in studies of Ca metabolism, the tooth is a very sensitive index of abnormalities in Mg deficiency.

It is interesting to contrast the effect of Ca and P deficiency on the dentine with those of Mg lack. In Ca and P deficiency, the dentine-predentine junction is very irregular, owing to malfusion of the calcospherites, and this may lead to the formation of interglobular dentine; in Mg deficiency, the dentine-predentine junction is unusually straight and regular and no calcospherites are seen, the dentine being very homogeneous in appearance. The predentine may be wide in both conditions, but, whereas in Ca and P lack, the measurement may be of all values up to 90μ , in Mg deficiency the width is usually a multiple of that normally found. The wide predentine in Ca and P lack never shows the striations which are such a conspicuous feature of the late stages of Mg deficiency. Moreover, the stratifications caused by Ca and P lack, when they are present, occur in the dentine and not in the predentine, and are due to malfusion of calcospherites, being in fact a species of interglobular dentine. The predentine step is peculiar to Mg deficiency; vascular inclusions, conspicuous abnormalities in Ca and P deficiency, have never

been noted in Mg deficiency; and, lastly, the odontoblasts do not shrink in Ca and P deficiency.

The recovery process, when the animal was returned to the adequate diet, is interesting as showing that structures already formed during the deficiency, apparently could not be changed, but were covered by normal tissues and finally lost at the distal end of the tooth; only the edge of the old predentine was calcified and that but to a slight extent. All the abnormalities gradually moved to the far end of the tooth, the structures laid down behind them being entirely normal. The predentine step and the wide predentine were fully recognizable even after 10 days on the adequate diet, though they were by then deeply buried under a layer of new dentine. The new dentine became gradually wider with successive days on the adequate diet until after 10 days, it and the predentine together were about 135μ wide. The odontoblasts had recovered their full height after 6 days on the adequate diet, but it was evident that those laid down during the deficiency were permanently abnormal, since many of them were included in the dentine and predentine. Also, the new predentine first laid down beyond the step was abnormally narrow and the full width was not attained till the animal had been 6 days on the diet. This corresponded with the complete recovery of odontoblast height and accounted for the fact that the dentine plus predentine width at 10 days was lower than normal.

The rate of movement of the predentine step corresponded well with the rate of tooth growth obtained by marking the tooth [Duckworth & Godden, 1940]. Between the second and eighth days on the adequate diet, the step had moved 2.4 mm. This was at the rate of 2.8 mm. per week, the other method yielding a figure of 2.9 mm.

The calcified granules in the enamel organ behaved likewise and would ultimately have been lost. No new ones were formed after the animal had been returned to the adequate diet.

Erdheim's classical work in 1906 showed that parathyroidectomy exerted a profound influence upon the histology of tooth calcification, and this was later found to be due to interference with Ca metabolism. In the case of Mg deficiency, no such correlation is known to exist with any endocrine gland. It is true that certain changes bear a superficial resemblance to those induced by hypophysectomy. But it is far too early to do more than comment upon this likeness and suggest it as a possible approach for further work. It can only be considered as very striking that a lack of Mg, which enters into the composition of the tooth in very small amounts, should exert such a marked histological effect.

SUMMARY

A series of characteristic changes occur histologically in teeth during acute Mg deficiency. The calcification rhythm is upset and adventitious calcification occurs in abnormal situations. After restitution of Mg in the diet, the normal calcification cycle is immediately resumed.

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EXPLANATION OF PLATES I-III

PLATE I

a, enamel organ. *b*, enamel. *c*, dentine. *d*, predentine. *e*, odontoblasts.

- Fig. 1. Longitudinal section of the basal end of the upper incisor of a 40-day stock rat ($\times 120$). This shows the normal structure of the predentine and dentine and also of the enamel organ.
 Fig. 2. Longitudinal section of the basal end of the upper incisor of a rat 6 days on the Mg-deficient diet ($\times 120$). The predentine step is clearly seen.
 Fig. 3. Longitudinal section of the basal end of the upper incisor of rat 6 days on the deficient diet ($\times 47$). Two steps can be seen in the predentine.
 Fig. 4. Longitudinal section of the basal end of the upper incisor of a rat 8 days on the deficient diet ($\times 47$). At the lower end, the predentine is normal in width. A predentine step is then seen with some stratification. This gives way to predentine of double the normal width, while more distally, the stratification reappears in the predentine.

PLATE II

- Fig. 5. Longitudinal section of the basal end of the upper incisor of a rat 9 days on the deficient diet ($\times 120$). A double layer of stratification in the predentine is clearly visible. The odontoblasts are atrophic.
 Fig. 6. Longitudinal section of the basal end of the upper incisor of an adult rat maintained on the deficient diet for several months ($\times 26$). Note the folding of the dentine, the almost complete atrophy of the enamel organ and the absence of organic enamel.

Fig. 7. Longitudinal section of the basal end of the upper incisor tooth of a 40-day stock rat ($\times 27$). This section shows the normal appearance of the structures at the basal end of the tooth.

Fig. 8. Longitudinal section of the basal end of the upper incisor of a rat 14 days on the deficient diet ($\times 120$). This section shows the inclusion of odontoblasts in the predentine and the calcified material in the enamel organ. The enamel organ is completely disorganized at this point.

PLATE III

Fig. 9. Longitudinal section of the basal end of the upper incisor of a rat 23 days on the deficient diet ($\times 120$). This section shows in more extreme form than does Fig. 8 the inclusion of odontoblasts in the predentine and the adventitious calcification of the enamel organ. A calcified matrix has also been laid down round the odontoblasts.

Fig. 10. Longitudinal section of the basal end of the upper incisor tooth of a rat 6 days on the deficient diet and 2 days on the Mg replacement diet ($\times 120$). Note the new dentine laid down as a deeply staining band, between the odontoblasts and the predentine formed during the deficiency. The new predentine is abnormally narrow.

Fig. 11. Longitudinal section of the mid part of the upper incisor of a rat 6 days on the deficient diet and 8 days on the Mg replacement diet ($\times 120$). Note the broad layer of new dentine and the normal predentine and odontoblasts. The step and predentine formed during the deficiency period are still distinctly visible.

Fig. 12. A. Longitudinal section of the distal part of the enamel organ in a normal rat ($\times 120$). B. Longitudinal section of the distal part of the enamel organ of a rat 23 days on the Mg-deficient diet ($\times 120$). C. Longitudinal section of the distal part of the enamel organ of a rat 54 days on a diet deficient in vitamin A ($\times 120$). (From a section by Irving & Richards [1939].) The similarity of the changes in B and C is very marked.

THE INFLUENCE OF DIETARY CALCIUM AND
PHOSPHORUS UPON TOOTH FORMATION

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MANY investigations have shown that the histological structure and the chemical composition of the continually growing rat's incisor are very susceptible to metabolic disturbances induced either by dietary derangements or by other experimental procedures. For example, the inorganic elements Ca, P, Mg and F, the vitamins A and D and the active principles of the parathyroid, pituitary and adrenal glands all exercise a profound influence on the calcification of the rat's incisor. (For recent reviews of the literature see Schour [1938] and Karshan [1938].)

Karshan [1930, 1931, 1933] and Karshan & Rosebury [1931, 1932, 1933] have studied the influence of the Ca : P ratio of the diet on tooth formation in some detail. Using diets with low Ca : P ratios and low Ca and P contents, these workers showed that the ash content and the histological appearance of the rat's incisor were significantly altered [Karshan, 1931; Karshan & Rosebury, 1933]. On the other hand, Karshan [1930, 1933] showed that a rachitogenic diet of the Steenbock and Black type with a Ca : P ratio of 7.27 did not lower the ash content of the rat's incisor, and Karshan & Rosebury [1932, 1933] found that the histological structure of these teeth was almost normal. Becks & Ryder [1931] state, however, that the minute structure of the incisors of rats given McCollum's rachitogenic diet 3143 which has a Ca : P ratio of approximately 4.0 is grossly abnormal, and Downs [1932], using twenty-four different diets in which the Ca : P ratio varied from 0.33 : 1 to 3.33 : 1, found that when the Ca : P ratio of the diet was very abnormal the histological appearance of the rat's tooth was impaired.

It is difficult to assess the value of this work, particularly as the observations of Becks & Ryder [1931] and Downs [1932] on the influence on the tooth of diets with high Ca : P ratios are contrary to those of Karshan and Rosebury [Karshan, 1931; Karshan & Rosebury, 1932], and it seemed to be desirable, before conducting extended studies on

tooth calcification, to obtain more accurate information regarding the influence exercised on the rat's incisor by diets of varying Ca and P contents and ratios under strictly controlled conditions. The experiments described in this paper were therefore undertaken. Groups of rats were fed on diets of known Ca : P ratio and Ca and P contents, the food intake being controlled within these groups so that the only variants were the actual intakes of Ca and P. The effects of the different diets were studied histologically and chemically in the teeth and chemically and radiologically in the skeleton. A preliminary account of this work has already been published [Gaunt & Irving, 1939].

METHODS

Three experiments were made using diets with Ca : P ratios of 0.5, 1.0 and 4.0, respectively. In each experiment four diets of the given Ca : P ratio were made containing different amounts of Ca and P. The diets were fed to groups of four rats taken at weaning (23 days), the calorie intakes of the rats in each group being equalized. When the animals were 70 days old they were killed. This age was chosen because it had previously been found that at 70 days the changes induced in the rat's incisor by an inadequate diet were most pronounced [Gaunt, Irving & Thomson, 1939]. In addition, as the rat's incisor replaces itself about every 40 days, all the tooth substance present at 70 days would have been formed whilst the animal was on the experimental diet.

Diets

The diets were constructed by adding Ca lactate and Na_2HPO_4 to a basal diet which had the following composition:

Corn starch	60	Filtered butter fat	12
Egg albumin	10	Salt mixture	3
Wheat gluten	10		

Yeast extract equivalent to 10 parts of dry powdered yeast.

The salt mixture had the following composition:

NaCl	55	KI	0.65
K citrate	250	MnSO_4 , anhydrous	0.11
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	60	CaI	0.055
Iron citrate	10	K alum	0.055

"Analar" salts were used as much as possible in making the salt mixture.

The yeast extract was prepared at room temperature in the following manner. 200 g. of dried powdered yeast were ground in a mortar with 800 c.c. of a mixture of equal volumes of absolute alcohol and distilled

water, transferred to a flask and shaken for 15 min. After filtering at the pump, the yeast was returned to the mortar, ground with a further 600 c.c. of the aqueous alcohol, shaken for 15 min. and filtered. A third extraction of the yeast was effected by repeating the process of grinding and shaking with another 600 c.c. aqueous alcohol. The volume of the combined filtrates (usually 1700–1800 c.c.) was measured. The equivalent of 100 c.c. of this extract (i.e. the extract from 10 g. of yeast) was used for each 95 g. of the other constituents of the diet. The inorganic phosphate was removed from the yeast extract by adding to the whole bulk 80 c.c. strong ammonia solution and a solution of 8 g. $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ in 20 c.c. distilled water. The resultant mixture was shaken, allowed to stand until the precipitate had settled, and filtered at the pump.

Two methods were used during the experiment for the incorporation of the yeast extract with the other constituents of the basal diet. In the first method, the corn starch, wheat gluten, egg albumin and melted butter-fat were worked in that order into the yeast extract by means of palette knives until a homogeneous paste was obtained. The paste was dried in a current of air, ground in a mortar and sieved. The dry diet contained about 10% moisture and no residual ammonia. In the second method, which was adopted in the latter part of the experiment because of its greater ease, the yeast extract was evaporated in a current of air to small bulk and the corn starch, egg albumin, wheat gluten and melted butter-fat worked in by hand. The diet prepared in this way was a fine, light powder and did not require grinding. By both methods of preparation the salt mixture was the last constituent to be added to the basal or the experimental diets.

The basal diet (plus the salt mixture) contained 0.028% Ca and 0.066% P. Calcium lactate B.P. and Na_2HPO_4 "Analar" were added to form the twelve diets used in the three experiments. These diets contained the following amounts of Ca and P:

Exp. no.	Diet no.	Ca %	P %	Ca : P ratio
1	1	0.08	0.08	1.0
	2	0.12	0.12	1.0
	3	0.20	0.20	1.0
	4	0.30	0.30	1.0
2	5	0.08	0.16	0.5
	6	0.12	0.24	0.5
	7	0.20	0.40	0.5
	8	0.30	0.60	0.5
3	9	0.32	0.08	4.0
	10	0.48	0.12	4.0
	11	0.80	0.20	4.0
	12	1.20	0.30	4.0

Analyses for Ca and P were made on the experimental diets before the requisite amounts of the salt mixture, which is itself free from Ca and P, were incorporated with them. These analyses were done merely to check the additions of Ca lactate and Na_2HPO_4 and the efficiency with which these salts had been mixed with the dry basal diet.

The diets were moistened with water and fed as a stiff paste.

The animals fed on the diets containing the largest amounts of Ca and P developed normally calcified bones and teeth, whilst three rats fed *ad lib.* on diet 4 for about 70 days increased in weight by 2.5–3.0 g. per day. It seems, therefore, that all the factors necessary for normal development are present in the basal diet, provided that sufficiently large amounts of Ca and P are incorporated with it.

Rats

Male, Wistar Institute, albino rats were used. They were taken at weaning (23 days) and divided into groups each consisting of four litter-mates. Four such groups were used in each experiment, one rat in each group receiving one of the four diets of the given Ca : P ratio. The daily food intake of each rat was measured and the food consumption of the four rats in each group was made equal. In general the appetite of the rat on the diet with the lowest Ca + P content governed the food intake of the group, but occasionally the rat with the second lowest Ca + P intake was the pacemaker. The animals were individually housed in steel-mesh cages, cotton-wool being used as bedding. Distilled water was given *ad lib.* The cages were all kept in the same room at a temperature of 68–70° F. The animals were weighed at weaning and thereafter daily. When 70 days old they were killed with coal gas and radiographed. After post-mortem examination, the lower jaw and the right leg were removed from the carcass for the chemical analysis of the lower incisors and the right femur, tibia and fibula, and the remainder of the skull was removed for the histological examination of the upper incisors.

Radiography

The extent of rickets in each rat was determined by comparing the X-ray picture presented by the upper end of the tibia with the radiographic scale of Bourdillon, Bruce, Fischmann & Webster [1931]. In this scale, in which an ascending order of units represents diminishing degrees of rickets, 0 indicates the presence of extreme rickets and 12 normal calcification.

Analyses

Diets. Analyses for Ca and P were made on each diet prior to the addition of the salt mixture. Samples of the diets were incinerated in silica basins; in the P determinations, the samples were moistened with 20% Ca acetate in order to prevent loss of phosphoric acid during the ashing. The ashed samples were dissolved in hot HCl, and analyses for Ca and P were carried out on the solutions by the methods of McCrudden [1909-10] and of Fiske & Subbarow [1925], respectively. It was found that when the salt mixture was present in the samples of diet used for analysis, the ash produced fused into the silica basins, making extraction difficult and rendering the basins unsuitable for further use. Hence the salt mixture was added to the experimental diets only after the analyses had been accomplished.

Bones. The right femur, tibia and fibula of each rat were cleaned mechanically. The length of the femur was measured and then all the bones were extracted with boiling alcohol and ether. The fat-free bones were dried at 100° and their ash content determined by heating them in an electric furnace at about 650° until they had constant weight.

Teeth. The lower incisors were scraped clean, extracted with alcohol and ether and dried at 100°. The dentine was separated from the enamel by the flotation method described briefly by Manly & Hodge in 1936 and later given in full by these authors in 1939. Analyses for ash were made, in the manner described above for the bones, on the dentine fraction only.

Histology

The skulls, after the lower jaw had been removed, were fixed in formol-saline and decalcified in formol-nitric acid mixture. They were then divided longitudinally and the posterior portion cut away and discarded. The anterior portions were passed through 5% sodium sulphate, washed in running water, dehydrated with increasing strengths of dioxan and embedded in paraffin. Longitudinal sections of the upper incisors were cut at 10 μ and stained with haematoxylin and eosin.

RESULTS

General appearance. All the animals lived to be 70 days old, except one group in the first experiment. In this group the animal with the lowest intake of Ca + P died of pneumonia when it was 49 days of age. The remaining three rats were therefore killed and the examination of the group conducted at this age.

In all three experiments there was a great difference in appearance between the animals on the low and those on the high intakes of Ca + P. Those on the lowest intakes were stunted, had staring fur and sat in a characteristic posture with marked curvature of the thoracic and lumbar regions. They were very sensitive to handling, and in several cases the post-mortem examination revealed the presence of small beads of callus on the ribs, indicating that fracture had occurred. As the Ca + P intakes rose the appearance of the rats improved.

In almost all the animals, excepting those given diet 4 *ad lib.*, the hair became coarse and patches of baldness developed on the thorax and around the mouth; in many the hair around the eyes became thin and inflammation and exudate formation occurred. These symptoms were always more pronounced in the rats on the lowest intakes of Ca + P. The cause of this phenomenon is obscure. Butcher [1939] has found that dietary restriction interfered with the second growth of hair, and Day & McCollum [1939] stated that in rats on diets low in P the coat roughens and the hair around the eyes becomes thin. It is possible that these factors operated to some extent in the present experiment.

Only two of the rats showed any naked-eye abnormality of the incisors. In these two litter-mates on diets with low Ca : P ratios, alternate bands of pigmented and unpigmented enamel were noticed. In all the other rats the incisors appeared to be normal.

At post-mortem examination the most outstanding feature was the beading of the ribs in the animals on the lower intakes of Ca + P. This was particularly marked in the first and third experiments, but was present to a much slighter degree in the second experiment. The incidence of the beading diminished as the Ca + P intakes rose, and none of the animals on the highest intakes showed any naked-eye disturbance of bone formation. No other striking abnormality was found. Several of the animals, irrespective of the diet they had eaten, had slight inflammation of the stomach and duodenum, but the authors have found that this is also common in stock rats.

Chemical and radiological results. The data obtained from one group of rats in each experiment are given in Table I. The results given by the other three groups in each experiment were very similar to these.

In all three experiments the ash content of the bone was lowest in the rat on the diet poorest in Ca and P, and as the Ca + P intakes increased it rose to a figure of between 57 and 62%. The normal figure for stock rats at about this age is 63%. Although the body weights of the four rats in each group were almost identical, the bone weights, bone-ash weights

TABLE I

No.	Diets		Total consumed g.	Animals										Width of predentine μ
	% Ca	% P		Wt. at weaning g.	Wt. at death g.	Wt. bone g.	Ash % bone	Degree of rickets	Length of femur cm.	Wt. of tooth g.	Wt. of dentine g.	Ash % dentine		
						Exp. 1, Ca/P=1								
1	0.08	0.08	384	44	113	0.2072	42.18	10	2.32	0.0810	0.0573	68.93	41*	
2	0.12	0.12	382	41	92	0.2173	50.12	0	2.30	0.0659	0.0435	68.05	43*	
3	0.20	0.20	384	42	110	0.2738	56.50	11	2.57	0.0951	0.0737	71.37	27	
4	0.30	0.30	380	43	99	0.3105	59.80	12	2.57	0.0920	0.0677	72.82	18	
						Exp. 2, Ca/P=0.5								
5	0.08	0.16	405	55	132	0.2968	43.63	7	2.73	0.0757	0.0403	66.76	81*	
6	0.12	0.24	409	56	127	0.3185	46.73	8	2.76	0.0694	0.0367	67.85	57*	
7	0.20	0.40	407	54	122	0.3848	54.25	9	2.84	0.0924	0.0696	70.55	26*	
8	0.30	0.60	408	54	132	0.4192	57.31	12	2.82	0.1043	0.0715	72.86	17	
						Exp. 3, Ca/P=4.0								
9	0.32	0.08	429	50	130	0.2877	26.69	2	2.31	0.1125	0.0845	70.05	53*	
10	0.48	0.12	431	52	145	0.3307	42.67	9	2.50	0.1164	0.0902	71.40	36	
11	0.80	0.20	431	53	149	0.4171	50.42	9	2.84	0.1175	0.0903	72.10	36	
12	1.20	0.30	431	48	141	0.4611	57.45	11	2.89	0.1103	0.0838	70.90	21	

* Vascular inclusions.

and the femur lengths increased as the Ca + P intakes rose. The influence of the diet in reducing the absolute weight of bone ash and the percentage of ash in the bone was most marked in Exp. 3, in which the Ca : P ratio of the diets was 4. This accords with the radiological observation that all the rats on diet 9 had rickets, two of them very severely indeed. In the first and second experiments, in which the Ca : P ratios were 1.0 and 0.5 respectively, ossification was considerably better, and no animal on either diets 1 or 5 presented an X-ray picture of severe rickets.

The findings in the teeth are in sharp contrast with those in the bones. The weights of the teeth were much less affected by the diets than were the bone weights, and the tooth weight was a more constant fraction of the body weight than was the bone weight. This relationship between body weight and tooth weight has also been noted by Gies & Perlzweig [1916] and by Karshan [1933]. The ash contents of the dentine in all three experiments showed considerably less change than did the ash contents of the bone. The greatest differences were found in Exps. 1 and 2, more particularly in the latter. In Exp. 3, where the Ca : P ratio was high and the effect of the lowest intakes of Ca + P on the bone ash was most marked, the dentine ash showed no change with different intakes of Ca + P.

Histological results. The histological appearance of the constantly growing incisor tooth of the rat is a very sensitive index of changes in calcification. In the sections examined in the present experiment, the calcified enamel had been lost during the histological preparation, and only the organic enamel, occupying the proximal third of the tooth, remained. Nothing abnormal was found either in this part of the enamel or in the enamel organ of any rat. On the other hand, the dentine of animals on the lower intakes of Ca and P showed all the changes associated with deficiency of these elements. Under normal conditions the dentine is first laid down as an uncalcified organic matrix, the predentine, which the present writers have found to vary in width from 16 to 20 μ in the basal part of the tooth. This matrix is laid down in a 24 hr. cycle. Minute calcospherites are then formed which fuse in a homogeneous manner with those already present in the dentine. When the supplies of Ca and P are inadequate the predentine becomes very wide, the calcospherites fail to fuse and therefore the dentine-predentine junction becomes irregular and interglobular spaces occur in the dentine. In very badly calcified teeth capillaries may be found as vascular inclusions in the predentine. In the present experiment the predentine width at the basal end of the tooth was measured with a micrometer eyepiece and any abnormality was noted.

The alterations in the minute structure of the teeth confirmed in general those found in their chemical composition. In all three experiments normal teeth were found in those rats with the highest Ca + P intakes. The calcification of the teeth became progressively worse as the Ca + P intakes fell. The most badly calcified teeth of all were found in the rats on the diets with the Ca : P ratio of 0.5, i.e. in Exp. 2. In this experiment the teeth of all the rats on diets 5, 6 and 7 had vascular inclusions and much interglobular dentine. The tooth of one rat on diet 5 had a predentine width of 90μ , and of another, listed in Table I, a width of 81μ . In Pl. I are shown microphotographs of the dentine of the four rats on diets 5-8 whose data are given in Table 1. These microphotographs show very graphically the poor formation of dentine on diet 5 and the gradual improvement that was obtained as the Ca and P contents of the diets rose.

At the low levels of Ca + P intake the teeth of animals on diets with the Ca : P ratio of 1.0 were better calcified than the teeth of rats on diets with the Ca : P ratio of 0.5. Apart from the group which had to be killed at 49 days of age, the teeth of all the rats on diets 1 and 2 had vascular inclusions; in the teeth of none of the rats on diet 3 were such inclusions present. The predentine widths, although greater than normal, were not so large as those of the corresponding rats in Exp. 2.

The teeth of the animals on the diets with the high Ca : P ratio were, at the lower intakes of Ca + P, much better calcified than those of the rats on the diets with the lower Ca : P ratios. In the teeth of only two of the sixteen rats examined were vascular inclusions present; they were found in one animal on diet 9 and one on diet 10. The teeth of the rats on the lower intakes of Ca + P contained much interglobular dentine. In general, the predentine widths were less than those of the corresponding rats in Exp. 2, but were a little greater than those of the rats in Exp. 1. However, the teeth formed in rats on the diets with the Ca : P ratio of 4.0 appeared in general to be slightly better calcified than the teeth formed in rats on diets with the Ca : P ratio of 1.0.

The results of the histological examination of the teeth compare quite well with those of the chemical analyses. At the lower intakes of Ca + P the grossly abnormal picture presented by the teeth of rats on diets with a low Ca : P ratio was accompanied by a low ash content of the dentine, whilst in rats on the diets with a high Ca : P ratio the more normal histological structure was accompanied by normal figures for the dentine ash.

The findings in the teeth were in striking contrast to those in the

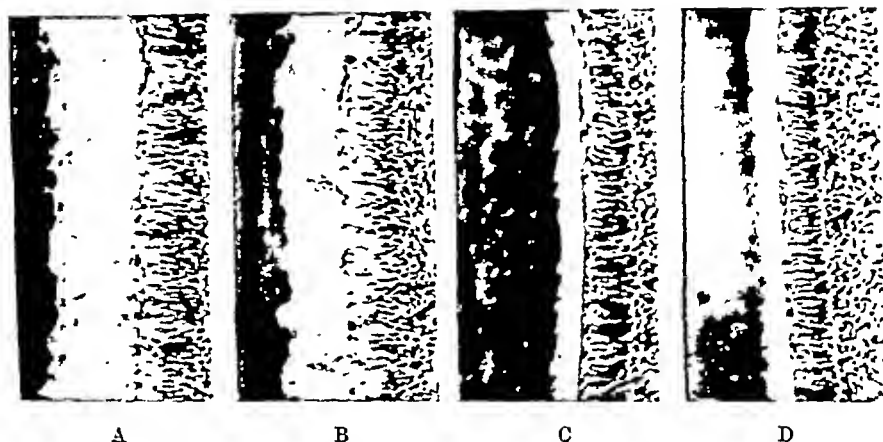


Fig. 1. Longitudinal sections of the dentine, predentine and odontoblasts from the basal end of the upper incisor teeth of the four rats in the second experiment the data of which are given in Table I ($\times 120$). *A* from the rat getting diet 5, *B* from the rat getting diet 6, *C* from the rat getting diet 7, *D* from the rat getting diet 8. Note the very wide predentine in *A* and the improvement in calcification as the Ca and P contents of the diet were raised. *D* is normal in appearance.

skeleton. The reduction in the ash of the dentine induced by the poorer diets was far less marked than in that of the bone. Furthermore, diets with low Ca : P ratios interfered with calcification of the tooth more than with calcification of bone, whilst diets with high Ca : P ratios had the opposite effect.

DISCUSSION

The results of these experiments have established the influence of the Ca : P ratio and the Ca and P contents of the diet on the calcification of the incisor teeth and of the skeleton of the rat, and have given evidence of the existence of a reciprocal relationship between the factors governing the calcification of the bones and that of the teeth.

Normal teeth were always produced between the Ca : P ratios of 4.0 and 0.5 when the Ca and P contents of the diets were sufficiently large. When the dietary content of the element present in the lesser amount fell below 0.3% the incisor teeth were abnormal, and the extent of the abnormality increased as the Ca and/or P contents of the diet fell further below this critical level. The influence of the Ca and P contents of the diet on the rat incisor increased as the Ca : P ratio fell from 4.0 to 0.5. For example, when the dietary content of the element present in the lesser amount was 0.08% the dentine ash values of the rats on the diets with Ca : P ratios of 4.0, 1.0 and 0.5 were approximately 71, 68 and 66% respectively.

Since the normal value for the ash content of the dentine is about 72%, these figures confirm the observation of Karshan [1930, 1933] that the tooth ash is unaffected by diets with high Ca : P ratios and of Karshan [1931] and Karshan & Rosebury [1933] that the tooth ash is lowered by diets with low Ca : P ratios. Confirmation of the histological results of Karshan & Rosebury is not so complete. Karshan & Rosebury [1933] noted that in rats on diets with low Ca : P ratios and low Ca and P contents the histological structure of the incisor was profoundly abnormal, the predentine being wide and many vascular inclusions being present in the dentine; this was also observed in the present experiment. It was found, however, in Exp. 3, where the Ca : P ratio of the diets was 4.0, that the histological structure of the rat's incisor was abnormal when the dietary content of P fell below 0.3%. This is contrary to the statement of Karshan & Rosebury [1932, 1933] that in rats on diets with high Ca : P ratios the histological structure of the incisors is practically normal. It is possible that the actual measurement of the predentine width, which was undertaken in the present experiment, records deviations from the normal more accurately than casual naked-eye examination.

It is interesting to note that the Ca and P contents of 1.22 and 0.32% respectively, and the Ca : P ratio of 4.0 in the diet used by Becks & Ryder [1931] (for the analytical figures see McCollum, Simmonds, Shipley & Park [1922]) are identical with the corresponding figures for diet 12 in the third experiment. Thus Becks & Ryder's diet should have produced normal teeth in rats. That this is not the case indicates that their diet was deficient in other respects.

The reciprocal relationship existing between the factors governing the calcification of the bones and of the teeth is well illustrated by the results obtained with the rats given the diets with the lowest Ca and P contents.

In the rats on the diet with the high Ca : P ratio the reduction in the ash of the bone was most marked (the lowest recorded value for bone ash was 25 %, which compares unfavourably with the normal value of 63 %), the degree of rickets most severe and the incidence of beading of the ribs highest; yet there was no reduction in the ash of the dentine, the histological picture of the tooth presented the least deviation from normal and the incidence of vascular inclusions in the dentine was almost nil. On the other hand, the diet with the low Ca : P ratio caused no marked rickets, the reduction in the bone ash was not so pronounced (the lowest recorded value was 44 %) and there was little beading of the ribs; however, the ash of the dentine was markedly lowered, the histological structure of the teeth was extremely abnormal and the incidence of vascular inclusions in the dentine was remarkably high. The diets with the Ca : P ratio of 1.0 occupied an intermediate position; their adverse influence on tooth formation was less pronounced than that of the diets with the Ca : P ratio of 0.5, and their general influence on bone formation greater.

These facts indicate that in some fundamental way there is a marked difference between the mineral metabolisms of bones and teeth. Since the chemical composition of the inorganic materials of bones and teeth are similar, it is difficult to see on theoretical grounds why this difference should exist. There are, however, other facts which indicate that this difference is real. Hauck, Steenbock & Parsons [1933] have shown that in chronic fluorine toxicosis the ash of the tooth is always lowered, although that of the bone is raised or lowered according to whether the Ca content of the diet is high or low respectively. Schour [1938] has recently summarized the very strong evidence that exists to show that the calcification of teeth, unlike that of bone, is an irreversible process, and the Ca and P in the teeth cannot be removed from them for use in

other parts of the body. Further investigations on this difference between the mineral metabolisms of bones and teeth are being conducted.

SUMMARY

1. With Ca : P ratios between 4.0 and 0.5, normal incisor teeth are only formed in the rat if the amount of each of these elements in the diet is at least 0.3%.

2. When the intake of Ca and P is not adequate, the calcification of the teeth is more disturbed by diets with the Ca : P ratio of 0.5 than by diets with Ca : P ratios of 1.0 or 4.0. On the other hand bone formation is interfered with more by diets with high Ca:P ratios.

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SURFACE AND FRAGILITY DIFFERENCES BETWEEN
MATURE AND IMMATURE RED CELLS

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IN the following experiments the fragilities and the effects of changing ionic environment on the electrophoretic speed of the two types of red cells previously found by differential sedimentation were compared. These red cell types are those in which aggregation tendency is small or entirely absent, and those in which it is normal or increased. The red cells with diminished aggregation tendency which form the upper zone or zones during sedimentation consist predominantly of reticulocytes and other immature forms [Stephens, 1938*a*,¹ *b*, 1939]. Although splenectomy or chronic partial loss of splenic function by exteriorization of the spleen affords a convenient experimental method of obtaining blood which will stratify during sedimentation, the effect may also be obtained with any blood containing appreciable numbers of immature red cells [Stephens, 1938*b*; Gripwall, 1938]. The electrophoretic relationships between reticulocytes and mature cells are similar in man, dogs, cats and guinea-pigs, so that the conclusions reached here need not be restricted to dogs. The red cells in the present experiments were obtained from a dog whose spleen had been exteriorized 6 weeks previously. The blood from such animals also exhibits stratified sedimentation, as already stated. The present animal had the added advantage that two upper zones formed, that is to say, three types of red cells distinguished by their sedimentation behaviour were available.

Red cells differentiated after 3 hr. sedimentation

The appearance after 3 hr. of the sedimenting column of blood diluted with one-fifth its volume of 3.8% sodium citrate is shown in Fig. 1. The same citrate concentration was used throughout the experiments. It

¹ Also reported in an oral communication to the Physiological Society, February 1938.

was difficult to portray the uppermost stratum photographically as there were very few red cells in it, although the numbers were adequate for electrophoretic measurements.

For convenience of reference the strata appearing after 3 hr. sedimentation are denoted by A, B and C zones, A being uppermost, as shown in Fig. 1. The B zone was exceedingly dense and, to the eye, much more sharply defined from the lower C column than appears in Fig. 1, which was photographed by transmitted light.

In the present case the triple stratification persisted for approximately 20 days and gradually merged into the commoner stratification with only one upper zone. This latter type of stratification continued for 13 months subsequently, and is still present. During the 20 days of triple stratification electrophoretic velocities of red cells from each zone were examined. The ionic composition of the suspending medium was varied in order to obtain information about the surface behaviour of the cells. Red cells from a normal unoperated dog were also examined.

Composition of upper zones

Stratified sedimentation means only that there are two types of red cells present, namely, those which aggregate and those which do not. Any form of red-cell damage which destroys the aggregation tendency of some of the cells also produces a blood which stratifies during sedimentation. This possibility has already been taken into account [Stephens, 1939], and must now be reconsidered because of the appearance during subsequent experiments of stratified sedimentation with the formation of an upper zone consisting of damaged red cells. During the present experiments, however, the B zone contained between 82 and 96% of reticulocytes estimated by single counts in which over 1000 cells were counted in wet mounts stained with brilliant cresyl blue. There nevertheless remains the possibility in some cases that two in every ten red cells

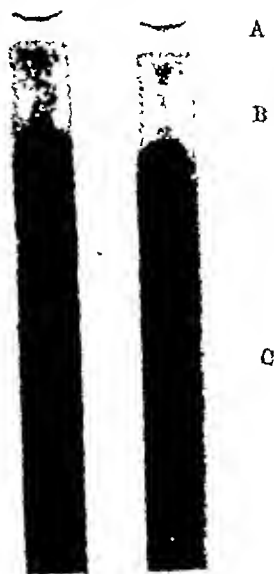


Fig. 1. Stratification appearances after 3 hr. Immature red cells are segregated in the A and B zones.

from this zone whose electrophoretic speed was being measured was not an immature red cell. Red cells may, however, be immature even after they have lost their reticulum. This was shown by other experiments where no reticulocytes could be demonstrated in upper zones which nevertheless exhibited the colour changes indicating rapid metabolic oxygen consumption characteristic of immaturity (see below). The probability, therefore, is that the percentage of immature cells present was higher than that shown by the reticulocyte count. Moreover, the individual electrophoretic speeds of all cells in a sample grouped closely. Individual migration times for A and B zone red cells varied at most by 2 sec. above or below the mean value which was of the order of 25 sec., and usually varied only by 0.5 sec. from this mean. The cells in the group could therefore be regarded as electrophoretically similar.

The C column samples, withdrawn from the bottom of the sedimentation tube, contained less than 6.5% of reticulocytes in all cases. The electrophoretic speeds of the great majority of red cells in this C column grouped much more closely. Reticulocyte counts were not made on the A zone because of the scarcity of cells, but reticulocytes were observed in it.

Accordingly, the B zone and C column are considered as immature and mature red cells respectively, the nature of non-reticulocyte cells composing the other zones being left undetermined, although the majority of these were probably immature red cells also.

Other evidence of immaturity of B cells

Although the C column remained bright red during the first 2 or 3 hr. of sedimentation, the B zone became dark lilac in colour, except for its extreme upper level which had access to atmospheric oxygen and which was also red. This colour change was very much more marked than that previously described [Stephens, 1939] and was apparent as soon as the B zone became segregated. It may be ascribed to rapid metabolic consumption of oxygen, which is greater in immature than in adult red cells [Warburg, 1909].

The low fragility of B zone cells, described later, may also be taken to accord with their immature character.

EXPERIMENTAL METHOD

The electrophoresis technique employed was that previously described [Stephens, 1939], employing a Brown & Broom flat electrophoresis cell of width/depth ratio 7 : 1. The measurements were made principally in 10% sucrose containing varying concentrations of added salts. The

experiments were performed at room temperature ranging from 17 to 19.5° C. and the results corrected to 18° C. on the basis of a 2% increase in electrophoretic speed per 1° C. rise of temperature.

Preliminary washing of the red cells with sucrose solution was avoided because of the surface changes thus produced [Monaghan & White, 1936], and because the aim was to examine surfaces as similar as possible to those in plasma. Suspensions of red cells had always a higher electrical conductivity than the suspending media alone, an effect which may have arisen partly from leakage of ions from inside the red cells or detached from their surfaces, but which was due predominantly to the small amounts of citrated plasma added with the red cells. It was thought better to accept this minor source of inaccuracy in the absolute although not in the relative values of the ionic concentrations of the suspending medium, rather than interfere with the red cell surfaces by washing them. The relative error arising from these adventitious sources of ions is greater for the readings corresponding to very low ionic concentrations than at other points. Because suspensions of red cells were always electrically conducting, the curves have not been continued to the ordinate of zero ionic concentration.

Preliminary experiments had indicated that the different electrophoretic speeds of cells from the various zones was caused by differential adsorption of ions. If this were the case, it would be desirable to ensure that the number of red cells and the volume of suspending medium in the electrophoresis cell were constant for all measurements because of the mass-substrate ("Bodenkörper") effect [Ostwald, 1927], which, in the present case, means that the amount of electrolyte adsorbed by unit mass of red cells is a function of the quantity of red cells present. Small quantities of an adsorbent (red cells) adsorb more electrolyte per unit mass from the same volume of suspending medium than larger quantities. The relationships may be expected to be those given by Kroecker adsorption curves [1892]. Here, no doubt, lies part of the explanation of the varying electrophoretic speeds measured when varying amounts of blood are introduced into the electrophoresis cell [Stephens, 1939] and also of the small diurnal variations in electrophoretic speed of red cells from the same animal when no precautions in this respect are taken.

The possible error arising from the "Bodenkörper" effect is to be distinguished from that resulting from the introduction into the electrophoresis cell of unequal amounts of citrated plasma with the red cells. The ideal here, as in all comparative electrophoretic measurements on red cells, is to secure uniform total red-cell surface and uniform citrated

plasma concentration in the same volume of suspending medium, and to keep the citrated plasma concentration as low as possible.

The C column was much more concentrated in red cells than the B zone. Accordingly the C zone samples were diluted with their own citrated plasma so as to yield suspensions of density as nearly as possible equal to that of the B zone. *Time considerations excluded more exact equalization* which was in any case unnecessary. Of these suspensions, 10 c.mm. were introduced separately into 6 c.c. of suspending medium in the electrophoresis cell where the final concentration was approximately 1×10^6 red cells per c.c.

Red cells from the A zone could not be brought to the same concentration as B zone cells in this way owing to their very low concentration. In this case and also with D zone cells described later, 10 c.mm. of the zone itself was introduced directly into 6 c.c. of suspending fluid in the cell. Any error thus introduced was probably small.

The citrate concentration introduced with the cells thus corresponded to a concentration of approximately 2.0×10^{-4} M. This was constant throughout the readings and was not corrected for in the results given. The concentration of plasma salts was probably similar.

Measurements made in the above way did not greatly differ from those when 10 c.mm. of the C zone cells were added without compensating for their greater concentrations, except in the very low salt concentrations of the suspending media. In previous experiments [Stephens, 1939] differences between the electrophoretic speeds of mature and immature red cells were observed when they were migrating side by side in the same suspending medium.

RESULTS

Isotonic sucrose—pH 7.4 buffer mixtures

Fig. 2a shows the migration times of four samples of red cells; top, A, middle, B, and bottom, C, layers from sedimentation tubes, and normal red cells. The curves represent the variation in electrophoretic speed when varying amounts of M/15 phosphate buffer, pH 7.4 [Hastings & Sendroy, 1924], are added to 10% sucrose solution in twice distilled water. The B zone here contained 82% of reticulocytes. The same results were obtained in a second such series of measurements, in which the B zone contained 87% of reticulocytes.

The results shown in Fig. 2a call for attention.

(1) Red cells from the top and middle zones have almost unaltered electrophoretic speeds over a wide range of ionic concentration of the

suspending medium. This resistance of the cells to surface alteration is remarkable.

(2) Red cells from the lower column and normal red cells vary in electrophoretic speed as the ionic concentration of the medium varies. Their speeds are equal in all media tested, and pass through a speed maximum corresponding to a molar salt concentration of approximately

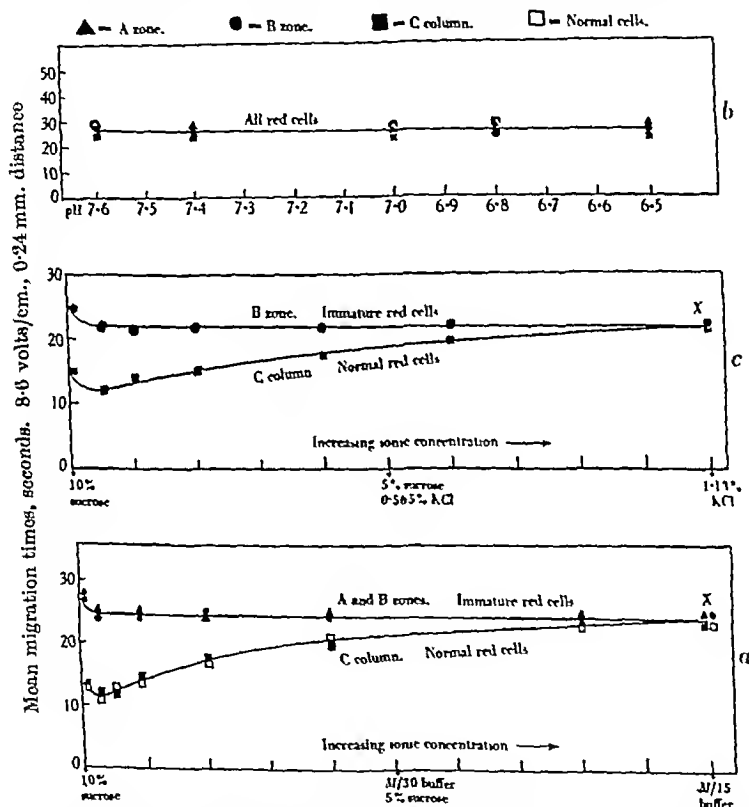


Fig. 2. Variation of the electrophoretic speed of mature and immature red cells with change in the ionic concentration of the suspending media.

M/300. The true position of this maximum probably occurs at a somewhat higher salt concentration because of the added citrated plasma and release of ions from the cells.

Series of buffers without sucrose

Because red cells migrate to the positive electrode, it is to be expected that positive ions will be predominant in determining the electrokinetic

potential of their surface, and therefore their electrophoretic speed. The preceding sucrose-buffer media contained varying concentrations of cations which may thus have been the essential factor.

It was of interest, therefore, to examine the behaviour of the red cells in *M/15* phosphate buffers varying from *pH* 6.5 to *pH* 7.6, sucrose being omitted. The results are shown in Fig. 2*b*, where it will be seen that all types of cells behave similarly within the limits of accuracy, and that there is a very small, approximately linear, decrease in the electrophoretic speed of all types when the *pH* of the suspending medium is decreased. The B zone contained 86 and 90% of reticulocytes during two series of readings in these buffers. Reticulocytes in the A zone were not estimated. The isoelectric point of the ghosts of normal red cells from the dog was shown by White & Monaghan [1936] to be *pH* 2.7, so that no great variation in electrophoretic speed in the above *pH* range is to be expected in the C column cells at least. It is impossible to determine electrophoretic speeds of intact red cells in media of high acidity because the value changes continuously with time.

The small, approximately linear, variation in electrophoretic speed of normal red cells from the dog with alteration of the *pH* of the suspending medium is similar to that found by Abramson [1930] for normal sheep red cells in the range from *pH* 4.5 to 6.5.

Isotonic sucrose—KCl mixtures

In each of the preceding buffers, although the hydrogen ion concentration varied, there was present a plentiful supply of other cations, potassium and sodium, so that the conditions may have corresponded to maintenance of the red-cell surfaces in the state corresponding to *X* in Fig. 2*a*, at which point also the electrophoretic speeds of all types of cells were the same.

Accordingly the cation (potassium) concentration of the milieu was again varied, sucrose being again necessary to maintain isotonicity. Fig. 2*c* gives the results in this respect using KCl, and again discloses a difference between the upper zone and lower main column cells. The B zone cells employed in two series of measurements contained 84 and 87% of reticulocytes.

Oliver & Barnard [1924], investigating the action of varying concentrations of NaCl on the electrophoretic speed of normal red cells from the rabbit, obtained a curve similar to the lower curve of Fig. 2*c* for KCl.

Isotonic salt solutions in isotonic sugar media

In order further to clarify the effects of varying ionic concentration, measurements of electrophoretic speed of B zone and C column cells were

made in media containing the electrolyte in isotonic concentration dissolved, not in water, but in isotonic glucose and sucrose solutions. Such solutions were, of course, hypertonic, but because the electrokinetic potentials arise at the red-cell surface and are apparently independent of the ionic composition of the red-cell contents, this procedure was permissible. In previous experiments [Stephens, 1939] crenation of red cells was found to be without influence on their electrophoretic speed.

The results are given in Table I in which the migration times are means of readings on twenty individual cells. The number of readings was restricted because the high electrical conductivity of the suspending media caused rapid heating. All individual migration times grouped within 2 sec., however. The values for pure sucrose and glucose media alone are also given, but the migration times of C column cells could not be satisfactorily determined in glucose without added electrolyte because of continuous speed changes with time. Sucrose media did not present this difficulty, although the range of speeds was wider in sucrose than in media containing salts.

TABLE I

Suspending medium	Mean migration times (sec.) for 0.24 mm. distance and 8.65 V./cm.	
	B zone	C column
1.13% KCl in 4% glucose	25.3	25.5
M/15 phosphate buffer in 4% glucose	21.9	22.0
1.13% KCl in 10% sucrose	31.8	32.1
0.85% NaCl in 10% sucrose	35.0	35.5
4% glucose alone	20.5	13.6-17.4 variable speed decreasing with time
10% sucrose alone	30.8	14.7

The values given in Table I again show that there is no difference in electrophoretic speed between the two types of red cells in media of high ionic concentration. The presence of glucose or sucrose does not alter this essential result. The absolute values of the electrophoretic speeds are seen to be determined by the nature of the ionic milieu and also by the nature of the sugar molecule present.

All the foregoing measurements thus show that electrophoretic speed differences between the different types of red cells become manifest as the ionic concentration of the suspending medium is reduced. Whether or not similar differences would also be revealed in media of ionic concentration higher than those shown in Table I and Fig. 1 remains to be determined.

As already stated [Stephens, 1939], washing of reticulocytes in two changes of isotonic sucrose solution buffered at pH 7.4 with 100 c.c. of *M*/15 phosphate buffer per litre, increases their electrophoretic speed to that of similarly washed normal red cells. The washing process here may involve other surface changes than the exchange of adsorbed ions, but the result is of importance in the interpretation of the phenomena.

RED CELLS DIFFERENTIATED AFTER 12 HR. SEDIMENTATION

It was possible to record photographically some of the interesting appearances after 12 hr. sedimentation. These are shown in Fig. 3 and further description is unnecessary. The same stratification was obtained in splenectomized animals, the band of leucocytes evidently corresponding to the leucocytosis which follows splenectomy.

In the region above the A and B zones residual discoidal and pineapple-shaped red cells were found suspended, and for convenience of reference these are designated as the D group. These residual cells were exceedingly few and could not be shown photographically. Electrophoretic speeds of all red-cell strata after 12 hr. in the sedimentation tube were measured in one suspending medium only: 10% sucrose to each 100 c.c. of which 10 c.c. of *M*/15 phosphate buffer were added.

Determinations were made on two different occasions separated by an interval of 3 days. The electrophoretic speeds thus found in zone C, and zones A and B (A and B have now intermixed) were the same as those found after 3 hr. The D group of cells had electrophoretic speeds lower than these.

The mean migration times for 8.6 V./cm. and 0.24 mm. migration

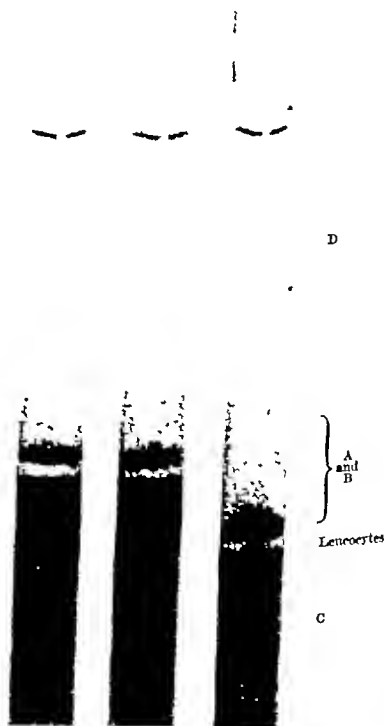


Fig. 3. Stratification appearances after 12 hr. Immature cells of A and B zones (now mixed) segregated above the band of leucocytes. (Blood has been removed from the C column of the tube on right-hand side of figure.)

distance are given in Table II. (Means of 20-40 individual cell migration times which grouped closely.)

TABLE II

	Mean migration time (sec.)
C	14.0
A and B now mixed	23.5
D	30.1

There is thus the approximately $1 : \frac{2}{3} : \frac{1}{2}$ ratio of electrophoretic speeds previously encountered [Stephens, 1939]. (From Fig. 2a it will be seen that the present sucrose-buffer suspending medium also gives a $3 : 2$ ratio of electrophoretic speeds for C and A or B zone red cells after 3 hr. sedimentation as reported previously.)

LOW FRAGILITY OF RETICULOCYTES

There is much controversy as to whether reticulocytes have greater or less fragility than normal adult cells. The conflicting authorities are discussed by Daland & Zetzel [1936], who consider that opinion is about equally divided. Subsequently, Dameshek & Schwartz [1938] found clear evidence that reticulocytes were less fragile than normal cells.

The possibility of deciding the question was presented when the B zone passed through the phase when it contained 96% of reticulocytes. These could thus be isolated. Fragility determinations showed quite definitely that the red cells of this upper zone were more resistant to hypotonic saline and other media.

(a) Hypotonic saline

Experimental. In order to avoid discrepancies arising from the difference in concentration of red cells in the upper zone and lower main column, the upper zone was pipetted off and centrifuged. A sample from the lower column was diluted with citrate until it contained the same number of red cells per unit volume as the upper zone, and this diluted suspension was then centrifuged. 50 c.mm. of each centrifuged deposit was then added to 2.5 c.c. of the sodium chloride solutions in the concentration series. Haemolysis was determined by naked eye inspection after 1 hr. This centrifuged deposit contained some citrate solution associated with it, but the amount was approximately the same in all measurements and was diluted with relatively large quantities of saline. It is difficult to see how this difficulty can rigorously be avoided in these experiments which lie at the lower limit of the NaCl concentration series. Because of this, therefore, the fragility values found cannot be taken as absolute. The results are given in Table III.

(b) *M/15 phosphate buffers ranging from pH 6.5 to 7.6*

After 12 hr. in these buffers haemolysis of normal and C column red cells commenced at pH 6.8 and was progressively increased in amount as the alkalinity of the series increased. Reticulocytes showed no haemolysis.

TABLE III

After 1 hr.	Exp. 1	
	Haemolysis	
	Begins % NaCl	Apparently complete % NaCl
Upper zone B (96 % reticulocytes)	0.1	(Not determined; incomplete in 0.1)
Lower main column C	0.25	0.1
Same specimens after 12 hr.		
Upper zone B	0.45	0.1
Lower main column C	0.45	0.15
Exp. 2		
After 1 hr.		
Upper zone B (84 % reticulocytes)	0.35	(Not determined; incomplete in 0.1)
Lower main column C	0.35	0.1
After 12 hr. (same specimens)		
Upper zone B	0.45	0.1
Lower main column C	0.45	0.15

(c) *Exposure for 48 hr. to large excesses of 3.8 % sodium citrate solution*

Such excessive dilution of normal red cells causes haemolysis after 6 hr. Fig. 4 is a photograph showing absence of haemolysis of reticulocytes after 48 hr. in 3.8 % citrate (tube C). This may be compared with the result for lower column cells (b) and whole blood from the general circulation (a) which have been only 12 hr. in the same citrate. The photograph was made on panchromatic film which renders the marked haemolysis in tubes (a) and (b) much less apparent than was actually the case.

This haemolysis produced by excess of citrate is probably caused by the alkalinity of this solution which was found by colorimetric methods to have a pH 7.7, and may thus be expected to haemolyse similarly to the alkaline buffers described above. Furchgott [1940] showed that red cells change in shape from disks to spheres and then undergo haemolysis in alkaline media, the effect being enhanced by removal of plasma. The familiar Gough transformation of red cells from disks to prolytic spheres when suspended in saline media [Gough, 1924; Ponder, 1925] was also shown by Furchgott to be dependent on the alkalinity caused by the glass slide and cover slip during the examination. The sphering in Furchgott's experiments apparently occurred immediately at pH 11 and the other data given by Furchgott suggest that the change may occur at lower pH after some time. This was in fact found to be the case and red cells from a normal dog were either lysed or converted into spherocytes

after suspension for $\frac{1}{2}$ hr. in isotonic saline brought to pH 8.2 by the addition of a few drops of $N/10$ NaOH solution. Blood from splenectomized or exteriorized spleen dogs introduced into such alkaline solutions behaved similarly, but even after 1 hr. there remained always a residuum of unaltered discoidal cells. Presumably these were immature red cells

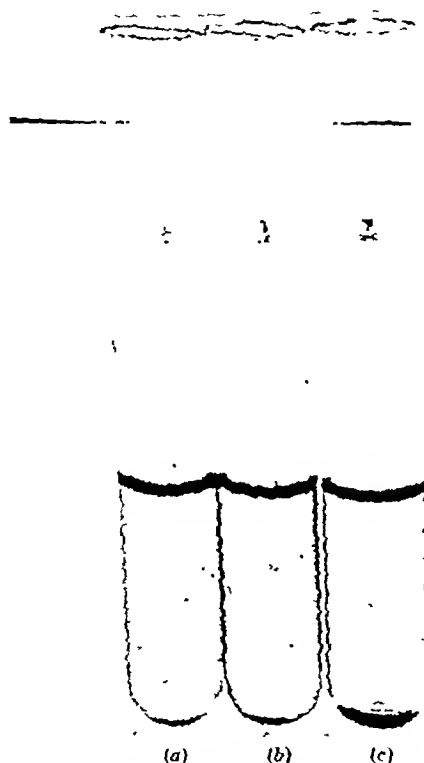


Fig. 4. Low fragility of reticulocytes (c), in an alkaline medium in comparison with red cells from the general circulation (a) and from the C sedimentation zone (b). The reticulocytes have been in the 3.8% Na citrate of pH 7.7 for 48 hr., the normal red cells for 12 hr. only.

which previous experiments [Stephens, 1939] have shown to occur in large numbers in such blood. Another method of isolating immature red cells is thus offered by their high osmotic resistance in alkaline media.

"Target" corpuscles

Barrett [1938] described a special form of erythrocyte possessing increased resistance to hypotonic saline. In wet films these corpuscles were bowl-shaped and when fixed in formaldehyde a protrusion in the

centre of the concavity produced a refraction of transmitted light such that a central dark shadow appeared. These so-called "target" corpuscles appeared in sickle cell anaemia and after splenectomy for other conditions, persisting for at least 3 months after removal of the spleen.

During the present work such cells were commonly seen in the electrophoresis cell when blood from splenectomized dogs was being examined. Such cells possessed electrophoretic speeds lower than normal, falling into the group with speed approximately two-thirds normal. They are therefore probably only immature cells, which may also have an increased resistance to hypotonic saline.

MECHANISM OF STRATIFICATION

Red cells segregate in the upper layers because they remain non-aggregated, the rate of fall of such individual cells being less than that of cells aggregated in rouleaux [Stephens, 1939]. In further observations it was found that diluting the blood with its own plasma obliterated the sharpness of the stratification so that the lower column merged diffusely into the upper zones. Similar diffuseness is frequently seen in anaemic bloods. The greater the red-cell concentration in a sedimenting specimen, the sharper was the demarcation between the various levels. This accords also with the similar effects of particle concentration on the sharpness of stratification of sedimenting suspensions of clays, which, like blood, become stratified when the particles are of different sizes [Tuorila, 1927].

The electrolyte concentration of the plasma plays an important part in the stratification. Variation of the quantity of isotonic sodium citrate used, or addition of isotonic saline or buffers, always altered the appearance of the strata. Usually the larger the amounts of salts added, the less distinct was the demarcation of the zones. Experiments so far indicate that there is an optimum salt concentration corresponding to a given red-cell population and red-cell concentration, for the development of stratification. Variations from this optimum either give no visible stratification or less clearly defined zones. Nasse [1836] found that addition of sodium chloride caused the appearance of stratification in bloods in which the phenomenon was otherwise absent. Enocksson [1931] also observed marked effects of varying salt concentrations on the sedimentation of red-cell suspensions. The fluctuations from day to day in the depth and density of upper zones, observed during the present experiments and also previously reported [Svedin, 1936; Stephens, 1939], are of interest here. All these effects are apparently related to differential ionic adsorption on red-cell surfaces as discussed later. The Kroecker

adsorption isotherms give the adsorption per unit mass of adsorbent from an electrolyte of initial concentration c , as equal to $c/m(1 - e^{-km})$, where k is a constant and m the total mass of adsorbent present [Ostwald, 1927; Buzágh, 1936]. There are thus reasons for expecting effects either of varying red-cell numbers or varying plasma salt concentration on sedimentation phenomena if adsorption of ions on the cell surface is the essential mechanism.

DISCUSSION

Red-cell surfaces

The electrophoretic measurements reveal a surface difference between three types of red cells, i.e.

(1) Red cells from the lower main column C. These have the same electrophoretic speed as normal red cells.

(2) Red cells from the upper zones A and B, which have equal electrophoretic speeds, less than those of the C column cells in the media examined. The more rapid sedimentation of B zone cells compared with those in the A zone probably arises from their greater density. There was no obvious difference in cell size between these two zones, and both consisted predominantly of single non-aggregating cells.

(3) Red cells from the upper zone D.

Adsorption of ions on red-cell surfaces

The essential point for consideration is the origin of the varying electrophoretic speeds in different media and of the differences between mature and immature red cells. Any effect of varying salt concentrations of the suspending medium on the Donnan equilibrium across the red-cell surface cannot be the cause of the electrophoretic speed differences, because White & Monaghan [1936] found that ghosts of red cells from the dog have the same speed as intact red cells when suspended in sucrose media buffered at pH 7.4. Similar identity of electrophoretic speed of ghosts and intact cells of man and other animals in a variety of media is reported by Abramson [1934], Byler & Rozendaal [1938], and Abramson, Furchgott & Ponder [1939].

The present effects may therefore be taken to be independent of the red-cell contents and to originate at the cell surface. Accordingly, they are attributed to the greater charge density of absorbed ions on the mature cell surfaces, an explanation which accords with the electrophoretic identity of both types of red cells when a sufficient concentration of ions is available to saturate the immature red-cell surface (i.e. at the point X, Fig. 2a, c). In media of this high ionic concentration all red

cells have the same electrophoretic speed. When this ionic concentration is reduced adsorbed ions escape less readily from the mature than from the immature red-cell surface so that the electrophoretic speed of mature cells is relatively greater. The shape of the curves relating electrophoretic speed of C column and normal cells with ionic concentration indicates an ionic adsorptive process. The curves are of the same form and the point of minimum migration time occurs quantitatively at approximately the same ionic concentration as with "inert" surfaces such as glass, graphite, collodion, etc. The salt concentration corresponding to minimum migration time has the same value, approximately $1 \times 10^{-2} M$, for all "inert" surfaces. This value is, moreover, independent of the nature of the salt in the suspending medium provided that the effective ion is monovalent [Abramson, 1934]. The effective ion is that with sign opposite to that of the migrating particle, in this case the positive ion. On theoretical grounds the influence of cations predominates with surfaces which migrate to the anode although anions must also be expected to be adsorbed.

The interpretation of present results may thus be clarified by converting the electrophoretic velocity values into the corresponding surface charge densities on the red cells. Abramson & Müller [1933], and Abramson [1934] have shown that σ , the surface charge density, is related to the electrophoretic velocity v by the equation

$$\sigma = 35000 \sqrt{c} \sinh \frac{4\pi\eta v}{2\beta D},$$

where c is the molar concentration of the salt solution, β has the value 0.25 V. at 18° C. and η and D are respectively the coefficient of viscosity and the dielectric constant at the interface.

The values of D and η at the interface are unknown, but, following Abramson and others, it suffices for present purposes to regard them as constant and as having the same value as in water. Accordingly, Fig. 5 shows the results corresponding to Fig. 2a, c when converted from electrophoretic speeds to surface charge densities. It is now seen from Fig. 5 that the surface charge on mature red cells increases more rapidly than on immature cells when the ionic concentration of the suspending medium is increased. This means that *ions are more readily adsorbed on mature than on immature cells*. It is to be noticed also that the σ - c curves for immature cells in KCl and in a medium containing a mixture of Na_2HPO_4 and KH_2PO_4 are almost identical.

Further consideration is necessary of the nature of the D group of red cells found in very small numbers after 12 hr. sedimentation. These

were a residuum left after the majority of cells in the A and B zones had sedimented. Their numbers were so very few that they may easily have escaped notice during the electrophoretic determinations after 4 hr. sedimentation in which 20–40 cells only were timed for each determination. Probably several hundred red cells would have had to be timed to have found them in the A and B zones at this stage. This D group may, however, be cells which have been damaged either in the animal's circulation or during the 12 hr. residence in the sedimentation tube, and

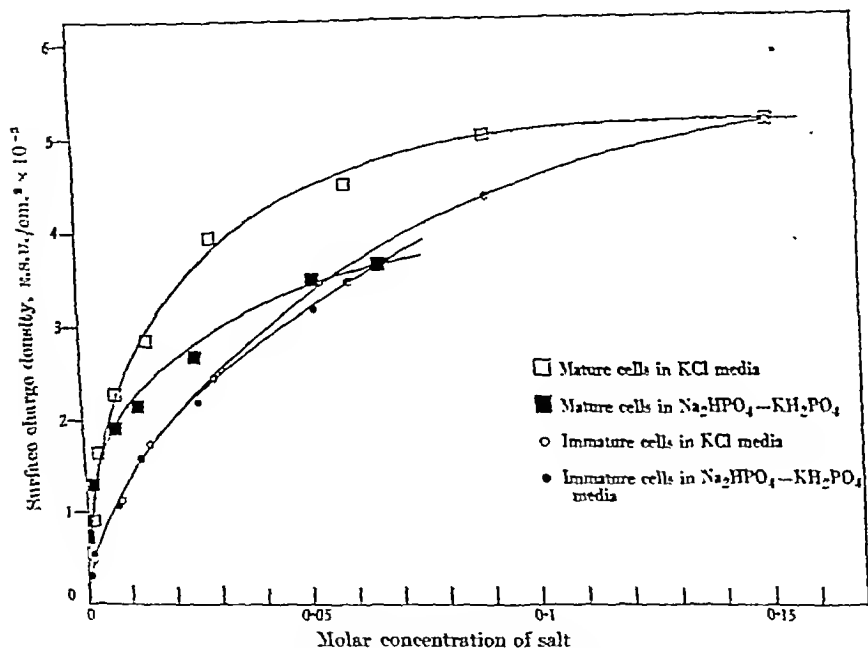


Fig. 5. Electrophoretic speeds of Fig. 2 expressed as surface charge densities.

which originally possessed the same electrophoretic speed as the A and B zone cells. Monaghan & White [1936] have shown that the surfaces of red cells deteriorate after standing one or two days, the change being accompanied by a reduction in electrophoretic speed. They attribute the change to the adsorption of protein by the surface, but it may arise from loss or exchange of adsorbed ions.

The extremely small surface alterations shown by upper zone red cells, chiefly reticulocytes, in ionic environments of widely different composition becomes less remarkable when the results are converted into the form shown in Fig. 5. The origin of the surface difference between

mature and immature cells requires attention however. The conception of the red-cell surface as a lipin-protein mosaic given by Abramson [1934] is helpful here. The effects may thus arise from differences in the membrane lipides. But they could also originate in differences, removable by washing, in the "rigid" ionic double layer of the membrane-plasma interfaces. The central fact, however, is that mature red cells adsorb ions with greater facility than immature cells; they thus have a higher electrophoretic speed unless the medium has an ionic concentration sufficiently high to cause equal adsorption on both types of cell. The phenomena are capable of adequate explanation on this basis, and the previous suggestion [Stephens, 1939] that differences in electrical conductivity of the surfaces might be concerned becomes unnecessary.

*Relation of ionic adsorption to red-cell aggregation and
sedimentation rates*

The familiar desolvation-aggregation of colloidal systems [Kruyt & Bungenberg de Jong, 1928]; the suggestion of Fåhræus [1929] that aggregation of normal red cells may be facilitated by similar surface dehydration; and the results of White & Monaghan [1936] confirming Fåhræus's suggestion, indicate that aggregation tendency of red cells in general is related with the degree of hydration of their surfaces.

The simplified explanation of red-cell aggregation and rouleaux formation given by Fåhræus may be developed to account for the complex effects of salts, and to explain how the red-cell lysosphere may be modified. Furthermore, the association of high electrophoretic speed with high aggregation tendency in mature red cells and of low electrophoretic speed with low aggregation tendency in immature cells requires explanation. White & Monaghan [1936] also observed, in a series of red-cell suspensions from different animals, that the order of increasing electrophoretic speed was also the order of increasing aggregation tendency.

Aggregation may be considered to arise from long range London-Van der Waals forces [London, 1930; Kallmann & Wilstaetter, 1932], and to be opposed by the electrokinetic potential and by the lysospheres which Ostwald & Haller [1929] showed to invest the surface of coarse suspended units. Thus a low electrokinetic potential or a thin lysosphere favour aggregation. Fixation of water molecules in lysospheres is opposed by their thermal agitation and by their tendency to form tetrahedral arrangements with one another as they do in free water [Bernal & Fowler, 1933]. In blood three systems compete for the anchorage of water

dipoles: red-cell surfaces, protein molecules, and salt ions. Formation of protein-ion-water complexes introduces a complication but there is evidence that plasma proteins are not normally adsorbed on red-cell surfaces [Abramson, 1934; White & Monaghan, 1936].

The phenomena here may be compared with the apparently parallel case of the adhesion of quartz particles investigated by Buzágh [1930*a, b*]. The thickness of the lyosphere surrounding the particle was found to be more important than the electrokinetic potential in determining adhesion. Moreover, at certain concentration ranges of KCl or NaCl, increase of electrokinetic potential was accompanied by increasing adhesion. Lyosphere thickness and electrokinetic potentials could either both increase simultaneously or vary in opposite directions with alteration in the salt concentration. Dehydrating agents such as alcohol or tannins increased the adhesion just as, on Fåhræus's theory, dehydration of red-cell surfaces by globulin, fibrinogen, gelatin, etc. promote their aggregation. The results indicated that the surface investment was a structure of ions and water molecules.

Similar relationships in the case of red cells would enable the occurrence of low electrophoretic speed with low aggregation tendency to be accounted for.

SUMMARY

1. Immature red cells have almost constant electrophoretic speeds over a wide range of ionic concentration of the suspending medium. Mature cells show greater variations in electrophoretic speed over the same range of ionic concentration of the suspending medium. In both cases, however, the effects quantitatively resemble those shown by "inert" surfaces under the same conditions.
2. The results are interpreted to mean that mature red cells adsorb ions more strongly than immature cells, under the conditions of measurement.
3. In media of high ionic concentration immature and mature red cells have equal electrophoretic speeds. Differences become manifest in media of low ionic concentration.
4. Reticulocytes clearly have lower fragility than normal mature cells, both in hypotonic saline and in alkaline media.
5. The method of stratified sedimentation enables non-aggregating red cells to be separated from those which aggregate. Both immature and damaged red cells are thus separated from normal cells.
6. Both the red-cell concentration and the salt content of plasma are factors in the stratification of sedimenting blood.

7. The aggregation and electrophoretic characters of red cells are compatible with the view that the cell membrane plasma interface is occupied by a lyosphere of ions and water molecules, and that the thickness of this zone rather than the electrokinetic potential is predominant in controlling aggregation.

In conclusion, I wish to thank Prof. H. W. Florey for his encouragement in this work, and the Medical Research Council and the Nuffield Trustees for personal grants.

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THE SOLUBILITY OF ACETYLENE IN BLOOD.

1. DETERMINATION OF ACETYLENE DISSOLVED IN BLOOD OR OTHER LIQUIDS

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THE acetylene method for determination of cardiac output seems to give very constant figures for a given individual under basal conditions, and the results obtained with Grollman's method seem also in most cases to agree well with the results obtained by methods based on Fick's principle [Grollman, 1932; Cooke & Priestley, 1937]. The use of the acetylene procedure in cases of heart failure in man, however, is based on the assumption that the solubility coefficient of acetylene in the blood of such cases is exactly known, and that the acetylene pressure found in the air of the rebreathing system agrees very accurately with the acetylene pressure of the arterial blood itself. It is found, however, that in some cases of congestive heart failure the number of corpuscles is increased; while in cases with rheumatic fever and other infections the number of corpuscles is decreased, and although the changes in the solubility coefficient resulting from changes in the proportion of corpuscles in the blood may be small, it is necessary to have accurate knowledge of the solubility coefficients in such cases to get correct figures for the heart output.

1. *Principles of determination of acetylene in blood*

In the manometric determination of the amount of a foreign gas of high solubility, present in a sample of blood, difficulty arises from the fact that extraction *in vacuo* liberates also the gases normally present in blood. During chemical absorption the pressure of the extracted gases is unavoidably increased and, consequently, some of the gas not

2. Determination $C_2H_2 + N_2$ pressure in blood

Two drops of caprylic alcohol are drawn into the capillary below the cup of the van Slyke chamber, then 0.3 c.c. of a 1% saponin solution, two drops of 1% potassium cyanide (to inhibit any possible carbaminase activity) and 5 c.c. of distilled water are added and freed from gas by extraction *in vacuo* as usual. The gas so liberated is then ejected, and the procedure is repeated two or three times. Next a part of the liquid is forced into the cup, leaving exactly 2 c.c. in the chamber. Then a sample of 2 c.c. of blood is delivered from the pipette into the extracting chamber as described by van Slyke and followed by 1 c.c. of the solution from the cup. The stop-cock of the chamber is sealed with mercury, the chamber is evacuated by lowering the levelling bulb, until the surface of the mercury is exactly at the 50 c.c. mark and the chamber is shaken for 3 min. The O_2 and CO_2 are then absorbed by a gas-free 20% solution of sodium hydrosulphite in *N* caustic soda containing also 2% of the sodium salt of anthraquinone- β -sulphonic acid [van Slyke & Neill, 1924]. For this absorption 4 c.c. of the reagent are placed in the cup, the pressure in the chamber is raised by opening the tap connecting it with the levelling bulb, which is hung in the lower bracket, and 2 c.c. of the reagent are immediately drawn into the chamber. Then the re-extraction *in vacuo* for 3 min. with shaking is carried out. This liberates acetylene and nitrogen only. The solution is now brought as quickly as possible to the 0.5 c.c. or 2.0 c.c. mark, and a reading of the manometer (P_1) and of the temperature is taken. The gases so liberated are ejected. During ejection some of the acetylene goes into solution again, and the ejection must be repeated after further extraction *in vacuo* for 1 min. at least once, and if large amounts of acetylene are expected it should be repeated twice. After the last ejection the mercury in the chamber is again brought to the 50 mark, and the liquid is extracted for exactly the same time as used for the re-extraction of C_2H_2 and N_2 (3 min.) and a second reading at the 2 or 0.5 mark of the pressure (P_2) is taken. The difference of temperature between the two readings should not exceed $1^\circ C.$; higher differences must be prevented by pouring hot or cold water into the water-jacket of the chamber.

3. Calculation

As the solubility coefficient of acetylene differs considerably from the solubility coefficient of nitrogen, the nitrogen correction cannot be subtracted from the percentage volume figures, calculated on the

assumption that all the gases extracted have the same solubility in the liquid. The calculation used by van Slyke for the indirect determination of O_2 and CO_2 in blood would not be accurate enough. The correct way of calculating the nitrogen correction is to find the nitrogen pressure which actually exists in the chamber while the reading P_1 is being taken. This pressure P_N is easily obtained by dividing the percentage nitrogen volume figure of the blood by a factor taken from Table I, and calculated from van Slyke's equation for nitrogen determination.

TABLE I. Factor (F_N) for calculation of the nitrogen correction (P_N) at different temperatures.

$$P_N = \frac{\text{volume per cent nitrogen of sample}}{F_N}$$

Temperature °C.	Sample 2 c.c. $S=7$ $a=0.5$	Sample 2 c.c. $S=7$ $a=2.0$
15	0.0317	0.1251
16	0.0315	0.1246
17	0.0314	0.1242
18	0.0312	0.1237
19	0.0311	0.1232
20	0.0309	0.1228
21	0.0308	0.1224
22	0.0306	0.1219
23	0.0305	0.1215
24	0.0303	0.1210
25	0.0302	0.1206

S = volume of liquid in the extraction chamber.

a = volume of gas at which reading of pressure (P) is taken.

Human blood normally contains 1.2 vol. % N [van Slyke & Neill, 1924] and the solubility coefficient at blood temperature is 0.01628. When acetylene mixtures are breathed, however, the nitrogen content of the blood becomes smaller, but, as the nitrogen solubility is small and the nitrogen content of the alveolar air during the heart output determinations mostly varies between 65 and 70 %, we can assume that the human blood used for acetylene determinations contains on the average 1.0 vol. % N. In cases where the nitrogen pressure of the gas mixtures used varies considerably from the average figure, or in experiments on different animals, the nitrogen correction must be calculated from the solubility coefficient for the blood used and the nitrogen pressure which exists in the air when the blood is in equilibrium with it, using the formula volume % $N_2 = \frac{p \cdot \alpha \times 100}{760}$. In Table II solubility coefficients of some of the samples of blood we used and for distilled water are given.

TABLE II. Solubility coefficients of nitrogen

Liquid	Temperature °C.	α
Water	20	0.01598
Blood: Man	37	0.01628
Cat	37	0.01628
Ox	37	0.01754

α = Bunsen solubility coefficient.

By dividing the N vol. % figure by the factor taken from Table I, one obtains the nitrogen pressure which exists in the van Slyke chamber during the acetylene determination (P_{N_2}).

The acetylene pressure ($P_{C_2H_2}$) can be calculated from the equation $P_{C_2H_2} = P_1 - P_2 - P_{N_2} - c$, where P_1 corresponds to the first, P_2 to the second pressure reading and c to the amount found by a blank determination.

For the determination of the c correction two drops of caprylic alcohol, 0.3 c.c. of the 1 % saponin solution, two drops of 1 % potassium cyanide and the amount of water necessary to bring the total volume of the solution up to exactly 5 c.c. are measured into the cup of the chamber and then extracted *in vacuo* with 3 min. shaking. Then all gas is ejected. This procedure is repeated twice. Then 2 c.c. of the gas-free reagent solution used for O_2 and CO_2 absorption are added and the blank determination is completed, using the same technique as for the acetylene determination itself. Readings after the extraction and after ejection and further extraction *in vacuo* are taken at the 2 c.c. or 0.5 c.c. mark. The pressure difference found gives the amount of the c correction. The value of the c correction, as we found in our blank determinations, ranges between 0.7 and 1.6 mm. using the 2 c.c. mark, and between 2 and 4 mm. using the 0.5 mark for the constant volume. But as the accuracy of the c correction is very important for calculation of accurate acetylene figures, the blank determination should never be neglected, and the reagent solution should be stored "gas-free" as described by van Slyke & Neill [1924].

For the calculation of the acetylene content of the blood in volumes % (vol. % C_2H_2) the general equation of van Slyke & Stadie [1921] can be used,

$$\text{vol. \% } C_2H_2 = P_{C_2H_2} \times \frac{0.1316 \times i \times \alpha}{\text{c.c. sample}} \times \frac{1}{1 + 0.0384 i} \times \left[1 + \frac{S}{A - S} \right],$$

where vol. % C_2H_2 = vol. % of acetylene; $P_{C_2H_2}$ = acetylene pressure as calculated from the equation $P_{C_2H_2} = P_1 - P_2 - P_{N_2} - c$; i = the reabsorption

correction; c.c. sample=amount of blood used for determination=2; a =amount of the constant volume used; S =volume of solution=7; A =the mark to which the mercury was brought for the extraction *in vacuo*=50; t =temperature of water-jacket during the determination; and $\alpha' = \frac{T\alpha}{760}$ (where α is the Bunsen solubility coefficient of acetylene, as determined by Winkler).

The reabsorption correction i is obtained by comparing the actual volume of acetylene extracted from an equilibrated sample of water, with the volume calculated from the solubility [van Slyke & Stadie, 1921].

An example will illustrate the calculation of i for a determination at the 0.5 mark. The acetylene pressure of the gas mixture equilibrated with water was 30.92 mm. Hg, and the acetylene vol. % figure calculated from this pressure was $\frac{30.92 \times 1.03 \times 100}{760} = 4.195$ vol. %. The acetylene amount found by determination in the water sample and calculated under the assumption $i=1$ was 4.04 vol. % and therefore the reabsorption was 3.695 vol. % of the total amount of gas liberated by the extraction *in vacuo*. The correction i for calculating the factor $F_{C_2H_2}$ at the constant volume 0.5 is therefore 1.03695. For the constant volume 2 c.c. no reabsorption was found ($i=1$), and we think that this is perhaps due to a slight depression of solubility by the alkaline solution from which the acetylene was liberated. The i correction for 0.5 should be determined for every apparatus and, therefore, we give in Table III only the figures of $F_{C_2H_2}$ for $a=2$, where $i=1$. The uncorrected figures for $a=0.5$ can be easily calculated by multiplying the factors for $a=2$ c.c. by 0.25.

TABLE III. Factor ($F_{C_2H_2}$) by which pressure of acetylene ($P_{C_2H_2}$) must be multiplied to obtain the vol. % of acetylene in the sample analysed

Temperature ° C.	15	16	17	18	19	20
$F_{C_2H_2}$	0.1489	0.1481	0.1470	0.1461	0.1452	0.1442
Temperature ° C.	21	22	23	24	25	
$F_{C_2H_2}$	0.1433	0.1424	0.1416	0.1407	0.1439	

Volume of sample = 2.0 c.c.

Volume of liquid in extraction chamber (S) = 7.0 c.c.

Volume of gas at which reading of pressure (P) is taken = 2.0 c.c.

Constancy of results in acetylene determination on blood or other fluids

In Table IV solubility coefficients, determined by the method described above, are given for acetylene in water, in blood of man and different animals and in ox blood in which the proportion of corpuscles to serum was varied. The results obtained by determination of acetylene in water samples agree within ± 0.1 vol. % with the acetylene figures

TABLE IV. Solubility coefficients of acetylene in different liquids

Liquid	Temperature ° C.	Found α_1	Distilled water α_2
Water	20	1.03	1.03
Blood: H. S.	37	0.751	0.747*
J. G. P.	37	0.747	—
Cat	37	0.744	—
Ox, no. 1	37	0.749	—
Ox, no. 2. Whole blood	37	0.739	—
Ox, no. 2.	37	0.737	—
66.7% corpuscles, 33.3% serum			
Ox, no. 2.	37	0.7357	—
33.3% corpuscles, 66.7% serum			

α_1 = solubility coefficient determined.

α_2 = solubility coefficients given for water by Winkler at 20° C. and by Grollman at 37.5° C.

* Determined at 37.5° C. by Grollman.

calculated from the acetylene pressure which was in equilibrium with those samples.

It would seem from these results that changes in the corpuscular content of the blood do not invalidate the results of determinations of heart output made by the acetylene method. The effects of changes in gaseous exchange in the lungs in patients suffering from congestive heart failure still requires investigation.

SUMMARY

1. The sources of error in the determination of cardiac output in man which may result from changes of the solubility coefficient of acetylene in blood are discussed.

2. A method for the determination of acetylene dissolved in blood and other fluids is described. It is based on the extraction of the blood gases and the acetylene *in vacuo*, absorption of oxygen and carbon dioxide, manometric measurement of the pressure of the re-extracted nitrogen and acetylene and of the pressure after ejection of the gases from the van Slyke chamber. For the nitrogen correction the nitrogen pressure, actually existing when the measurement of the nitrogen and acetylene pressure is taken, is calculated. The method is accurate within the range ± 0.1 vol. %.

3. The solubility coefficients of acetylene in water, human blood and the bloods of different animals are given.

4. The solubility coefficient of acetylene in blood remains remarkably constant when the ratio of corpuscles to serum is changed.

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BRONCHOMOTOR RESPONSES TO STIMULATION OF THE STELLATE GANGLIA AND TO INJECTION OF ACETYLCHOLINE IN ISOLATED PERFUSED GUINEA-PIG LUNGS

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It has previously been reported [Hebb, 1939] that the bronchoconstrictor response to acetylcholine or to stimulation of the stellate ganglia can be reduced or suppressed by administration of ergotoxine. Investigation of this problem has since been continued, and it is now proposed to give a full account of the results obtained in the entire series of experiments.

METHODS

Forty-three experiments were performed on the isolated perfused lungs of guinea-pigs bred from the same strain and fed on a uniform diet. The perfusion technique described by Dale & Narayana [1935], Daly, Peat & Schild [1935] and by Petrovskaja [1939] has been adopted. In certain respects the procedure has been modified in order to prevent the early onset of "lung rigidity", a condition to which the perfused guinea-pig lungs are extremely susceptible. To all save six animals, adrenaline was given by subcutaneous injection an hour before death. Immediately after each animal had been killed (by a blow on the head) tracheotomy was performed and positive pressure ventilation begun. Cannulae were tied into the pulmonary artery and left auricle. Then the pulmonary blood vessels were washed through with perfusion fluid, the heart ventricles were fixed into a clamp, and perfusion was begun. During the experiment the animal was kept in a closed chamber (temperature 38-40° C.), where the lungs were maintained under positive pressure ventilation by means of a micro-pump similar to that described by Daly [1937]. The intrapulmonary pressure during a short period of the inflationary stage of each respiratory cycle was recorded on the kymograph by a water pressure-volume recorder.

Two kinds of perfusion fluid have been used: (1) low calcium hypertonic Tyrode solution recommended by Daly *et al.* [1935], and (2) heparinized guinea-pig's blood diluted with one or two volumes of 0.9% sodium chloride solution. The lungs were perfused at constant pressure by means of either one of two systems:

(1) "Open" perfusion in which the fluid was not recirculated. Tyrode solution was continuously supplied from a flask (37–38° C.) connected directly to the pulmonary arterial cannula, the perfusion pressure being determined by the relative levels of the cannula and the flask.

(2) "Closed" perfusion in which a given volume (20–25 c.c.) of either Tyrode solution or dilute heparinized blood was recirculated by a micro-perfusion blood pump [Daly, 1937] which was adapted so that the inflow into the pulmonary artery was maintained at constant pressure (for description, see Petrovskaja [1939]).

The venous outflow rate was measured in all experiments by means of a drop recorder.

Drugs were administered either by injection into the pulmonary arterial cannula or by adding them in known concentrations to the perfusion reservoir. The following have been used in this investigation: acetylcholine (Roche Products), adrenaline with 0.5% chlorotone (Parke Davis), atropine hydrochloride (B.D.H.), ergotoxine ethanesulphonate (B.D.H.), heparin (Jorpes 1.0% solution), nicotine (B.D.H.) and physostigmine (B.D.H.).

The stellate ganglia were exposed from the ventro-lateral aspects. Each ganglion was freed from its lateral connexions without disturbing its connexion with the rest of the sympathetic chain (except in some control experiments in which the chain was cut below the level of the stellate ganglion) and without injury to the tissue on its medial border. Then, while the ganglion was lifted slightly by means of a blunt hook inserted from the lateral side, shielded electrodes were slipped into place so that their tips came into contact with the ganglion but did not touch any other structure. For the electrical stimulations a Palmer induction coil was used. Each stimulation was applied for 2 or 3 sec.

RESULTS

(1) *Pulmonary responses to stimulation of the stellate ganglia*

Broncho- and vaso-motor responses to electrical stimulation of the stellate ganglia were observed in twenty-five experiments performed on isolated perfused lungs of guinea-pigs. In each experiment it was found

that such stimulation produced bronchoconstriction. This response was marked by a sharp rise in intrapulmonary pressure which, beginning with the onset of the stimulus, usually reached its maximum within 30 sec. and then began slowly to subside (Fig. 1B). The rise in intrapulmonary pressure, denoting bronchoconstriction, occurred both with separate and

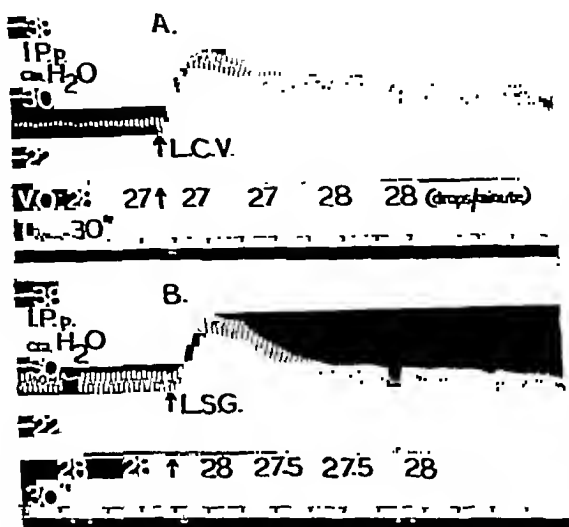


Fig. 1. 13. vi. 39. Guinea-pig 72. ♀; 650 g. Pretreated with adrenaline (0.25 mg. subcutaneously). Lungs perfused with hypertonic glucose-Tyrode solution in closed circulation at initial pressure of -5 cm. perfusate. Reading from top to bottom the four tracings are: the intrapulmonary pressure (I.P.p.); venous outflow rate (V.O.), as registered by the drop recorder (the figures given represent the number of drops per minute); time signal set at 30 sec.; and the signal line. At A (12.03 p.m.) the peripheral end of the cut left cervical vagus was stimulated for 3 sec. with the secondary coil set at 7 cm. At B (12.12 p.m.) the left stellate ganglion was stimulated for 3 seconds at coil distance 7 cm. (No other stimulus was applied between A and B.)

simultaneous stimulation of the right and left ganglia and with stimulation of the thoracic sympathetic chain immediately below the lower pole of the stellate ganglion.

These results are in agreement with those of Binger, Gaarde & Markowitz [1931], who, in a study of reflex bronchomotor phenomena in the guinea-pig, came to the conclusion that only a part of the efferent bronchoconstrictor nerves supplying the lungs are carried by the vagi, and suggest that the remainder are derived from the thoracic sympathetic nerves. The observations cited above are evidence in favour of the correctness of this assumption. Moreover, it has been shown in experi-

ments such as that illustrated in Fig. 1, that the bronchoconstriction produced by stimulation of the stellate ganglia is of the same order as that produced by stimulation of the peripheral ends of the cut cervical vagi. In other experiments of a preliminary nature, evidence was obtained indicating that at least some of the bronchoconstrictor fibres excited by stimulation of the stellate ganglia proceed to the ganglia from the upper dorsal region of the spinal cord and thence are distributed to the lungs. The peripheral distribution of these fibres has not been studied.

At this point the problem of immediate interest was to determine whether the sympathetic bronchoconstrictor nerve fibres are cholinergic or adrenergic. In this connexion it is worth noting that Thornton [1939] has obtained evidence indicating that in the guinea-pig the bronchoconstrictor fibres of the vagi nerves are cholinergic, while Petrovskaja's [1939] experimental results are not incompatible with the thesis that at least some of the same group of nerve fibres are adrenergic. In the present study, the problem of the sympathetic bronchoconstrictor nerves has been attacked by studying the effects produced by various drugs, including eserine, atropine, ergotoxine, nicotine and adrenaline, on the bronchomotor responses to stimulation of the stellate ganglia. The results of these pharmacological tests were as follows.

Comparison of the pulmonary responses to stimulations of the stellate ganglia applied before and after administration of eserine (perfusion concentrations = 1:100,000 to 1:500,000) showed that the bronchoconstrictor responses during eserine perfusion were either greater in intensity (seven experiments) or longer in duration (six experiments) than in the control observations. In four experiments, one of which is illustrated in Fig. 2, these two effects were combined. In Fig. 2 it will be seen that the stimuli applied at D and at G were of the same strength but that the second stimulus applied during eserine perfusion produced bronchoconstriction which was 50 % greater than that registered at D. It should be added, since it is not clearly shown in the figure, that the contraction period at G was three times longer than the contraction period at D. The potentiation here demonstrated derives additional significance from the fact that previous to the administration of eserine the preparation had exhibited a steady loss in sensitivity, as shown by the diminishing responses to successive stimuli.

Atropine usually produced a fall in intrapulmonary pressure. In seven preparations where the action of the drug was studied, it was found that atropine (in perfusion concentrations of 1:2000 to 1:70,000) either diminished, or suppressed, or reversed the bronchoconstriction

which was normally produced by stimulation of the thoracic sympathetic nerves. Suppression of the response was the most frequent sequel to administration of this drug (see Figs. 3, 4). In certain of the atropinized animals, however, stimulation of the stellate ganglia produced weak bronchodilatation.

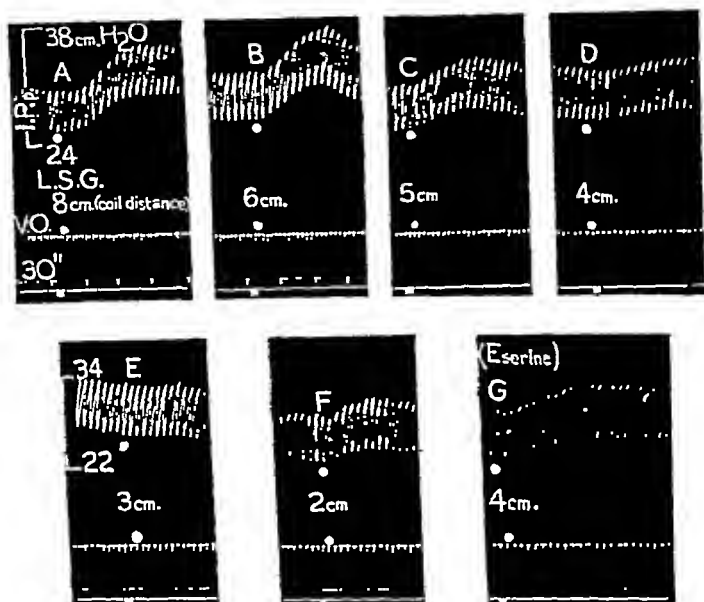


Fig. 2. 21. xi. 38. Guinea-pig 47. ♂; 900 g. Pretreated with adrenaline (0.25 mg. subcutaneously) and heparin (9 mg. intraperitoneally). Lungs perfused with hypertonic Tyrode in open perfusion system at initial pressure of +6 cm. perfusate. The tracings are as in Fig. 1. A-F (3.40-4.05 p.m.), stimulation of the left stellate ganglion at the coil distances shown during Tyrode perfusion. G, 4.13 p.m., stimulation of the left stellate ganglion during eserine-Tyrode perfusion (1:500,000).

Administration of ergotoxine was found to have effects similar to those produced by atropine in that it reduced or suppressed bronchoconstrictor responses to sympathetic nerve stimulations (see Fig. 3). Doses of ergotoxine varying from 300 μ g. to 1 mg. (perfusion concentrations = 1:20,000 to 1:100,000) produced suppression of the response for periods varying from 25 min. to 1 or 2 hr. (nine experiments).

In view of the observation made in the above experiments that the bronchoconstrictor response could be suppressed either by atropine or ergotoxine, it was not at all clear what type of nerve fibres were involved. It was hoped that some light on the problem might be gained by a study

of the responses of nicotinized lungs. In seven experiments it was found that initial injections of nicotine (50–100 μ g.) produced marked bronchoconstriction and rendered the lungs insensitive to further injections of the same drug. However, the lungs continued to respond normally to stimulation of the stellate ganglia, the stimulations producing in the

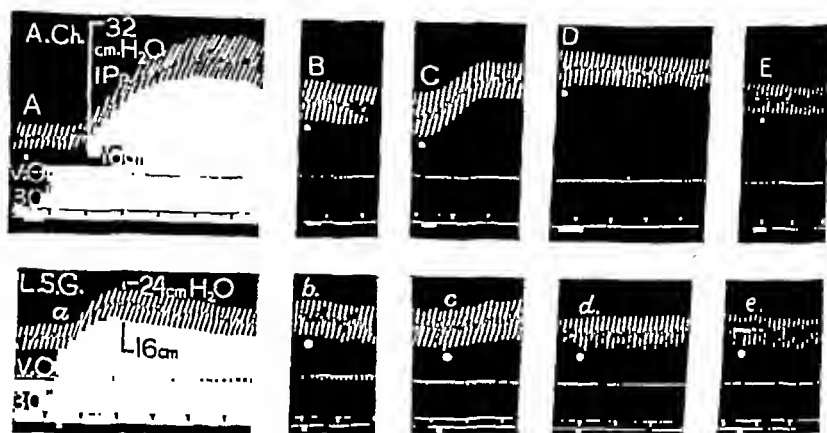


Fig. 3. 24. iv. 39. Guinea-pig 63. ♀; 460 g. Pretreated with adrenaline (0.25 mg. subcutaneously) and atropine (0.1 mg. subcutaneously). Lungs perfused with hypertonic glucose-Tyrodé in closed circulation at initial perfusion pressure of +3 cm. perfusate. Tracings are as in Fig. 1. The order of events in this experiment was as follows:

- | | |
|---|---|
| A. 12.44 p.m. 20 μ g. ACh. | a. 12.35 p.m. left stellate ganglion, coil distance 1 cm. |
| 12.55 p.m. 300 μ g. <i>ergotoxine</i> injected. | |
| B. 1.06 p.m. 20 μ g. ACh | b. 1.09 p.m. left stellate ganglion, coil distance 1 cm. |
| C. 1.55 p.m. 20 μ g. ACh. | c. 1.47 p.m. left stellate ganglion, coil distance 1 cm. |
| 2.12 p.m. 200 μ g. <i>atropine</i> injected. | |
| D. 2.23 p.m. 20 μ g. ACh. | d. 2.26 p.m. left stellate ganglion, coil distance 1 cm. |
| 2.32 p.m. 100 μ g. <i>atropine</i> injected. | |
| E. 2.40 p.m. 20 μ g. ACh. | e. 2.37 p.m. left stellate ganglion, coil distance 1 cm. |

(Each stimulation of the stellate ganglion lasted approximately 3 sec.)

nicotinized preparations bronchoconstriction of the same order as that observed in the untreated lungs (see Fig. 6). It seemed clear, therefore, that at least a majority of the sympathetic bronchoconstrictor fibres do not relay within the area supplied by the pulmonary perfusion system. In view of this conclusion, the earlier finding that eserine potentiates the bronchoconstrictor response points to the probability that cholinergic post-ganglionic neurones are involved in that response.

There were other possibilities to be considered as well. Petrovskaja [1939] observed in some of her experiments on guinea-pigs that, within the same experimental period, stimulation of the cervical vagosympathetic nerves, and injection of adrenaline separately, gave rise to bronchoconstriction; and that both effects could be reversed or abolished by ergotoxine. Accordingly, she suggested that there were adrenergic bronchoconstrictor fibres in the cervical vagosympathetic nerve bundles.

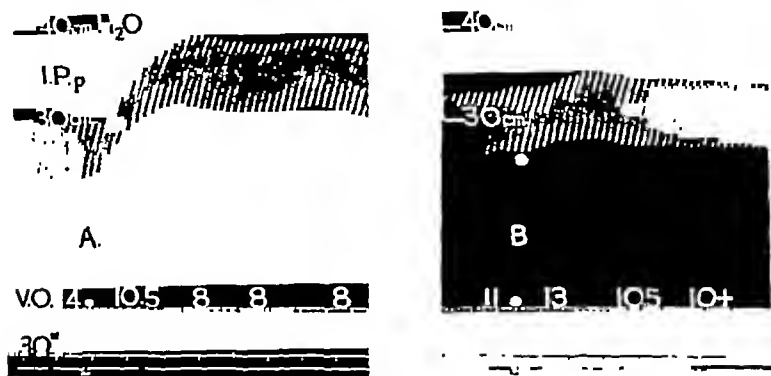


Fig. 4. 7. xi. 38. Guinea-pig 44. ♂; 700 g. Pretreated with adrenaline (0.25 mg. subcutaneously). Perfused with hypertonic Tyrode in open perfusion system at initial perfusion pressure of ± 5 cm. perfusate. Tracings as in Fig. 1. A, 1.37 p.m., stimulation of left stellate ganglion at coil distance 5 cm. for 3 seconds during eserine-Tyrode (1 : 500,000) perfusion. B, 1.56 p.m., the same stimulus repeated during atropine-Tyrode (1 : 10,000) perfusion. The perfusion fluid was changed from eserine to atropine-Tyrode at 1.47 p.m.

A similar argument might apply to the sympathetic bronchoconstrictor fibres but for one circumstance: it has been observed in the present experiments that, at a time when stimulation of the stellate ganglia caused bronchoconstriction, injections of adrenaline (1–20 μ g.) invariably produced bronchodilatation. Thus the results obtained with adrenaline injections lent no support to the view that the sympathetic bronchoconstrictor nerves may be adrenergic.

Injected adrenaline in many preparations produced a marked change in the responses of the lungs to subsequent stimulations of the stellate ganglia. Small doses (1–10 μ g.) injected into the pulmonary artery usually produced depression of the bronchoconstrictor effect for a short period (up to 20 min.) during which nerve stimulations either produced

no bronchomotor change or caused weak bronchodilatation as is illustrated in Fig. 5. When the lungs were perfused with adrenaline in larger concentrations (1–4 $\mu\text{g.}$), as was the case in four experiments, the largest

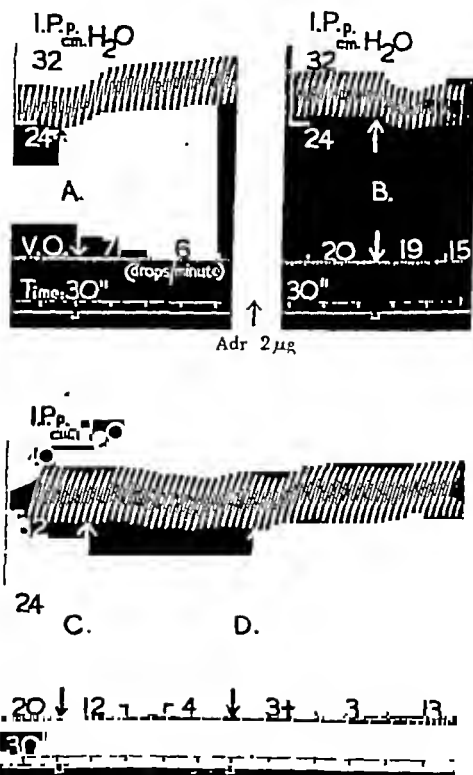


Fig. 5. 18. xi. 38. Guinea-pig 46. ♂; 817 g. Pretreated with adrenaline (0.25 mg. subcutaneously). Lungs perfused with hypertonic Tyrode in open perfusion system at initial pressure of +6 cm. perfusate. The tracings are in the same order as in Fig. 1. A, 12.26 p.m. Stimulation of both stellate ganglia, coil distance 8 cm. B, 12.45 p.m. Stimulation of both stellate ganglia, coil distance 7 cm. C, 12.56 p.m. Stimulation of both stellate ganglia, coil distance 5 cm. D, 12.57 p.m. Stimulation of both stellate ganglia, coil distance 5 cm. Between A and B, at 12.40 p.m., 2 $\mu\text{g.}$ adrenaline was injected into pulmonary arterial tubing.

bronchoconstrictor responses observed during a period of 2 hr. or more were so slight as to be just perceptible. This is in line with the results of Cordier & Magne [1927], who found that in the guinea-pig administration of adrenaline depressed the activity of the vagal bronchoconstrictor nerve fibres.

In the same study Cordier & Magne found that stimulation of the thoracic sympathetic nerves occasionally produced weak bronchodilatation, this result being regarded by them as evidence that there are sympathetic bronchodilator fibres innervating the guinea-pig lungs. To some extent that view is confirmed by two observations which have

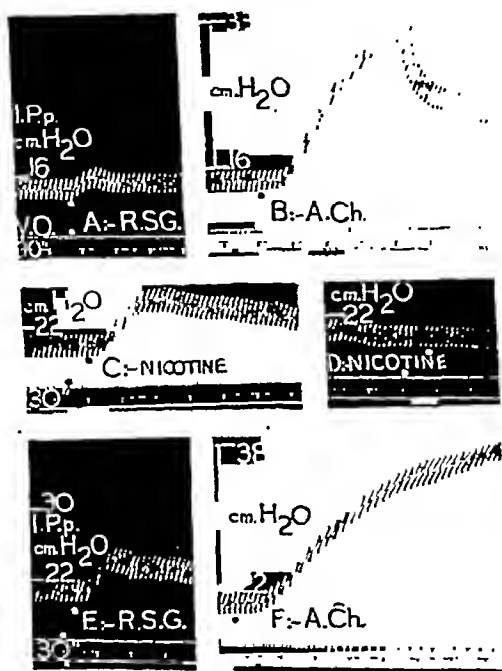


Fig. 6. 20. v. 39. Guinea-pig 69. ♀; 560 g. Pretreated with adrenaline. Perfused with hypertonic glucose-Tyrode in closed circulation at initial perfusion pressure of +4 cm. perfusate. Tracings as in Fig. 1. At A (12.19 p.m.) and E (1.49 p.m.) the right stellate ganglion was stimulated for 3 sec., at coil distances of 5 cm. and 3 cm. respectively. At B (12.26 p.m.) and at F (2.18 p.m.) 10 μ g. of ACh. were injected. At C (12.41 p.m.) and at D (12.49 p.m.) 50 μ g. of nicotine were injected.

already been mentioned in the present communication: one, that small doses of adrenaline may reverse the normal bronchoconstrictor response so that bronchodilatation occurs instead (cf. Fig. 5 A, B); and two, that with injection of atropine a similar reversal occurred in some preparations. It should be added, however, that Cordier & Magne did not find in any of their experiments that stimulation of the sympathetic nerves produced bronchoconstriction. This negative result may perhaps be

explained by the fact that the authors used anaesthetized (urethane) guinea-pigs for their experiments.

In response to stimulation of the thoracic sympathetic in guinea-pigs anaesthetized with nembutal, the only effect on the lungs observed by Dale & Narayana [1935] was vasoconstriction in one out of a total of four experiments. Since the effect was obtained in the absence of any bronchomotor change, the experiment may be regarded as evidence of the occurrence of pulmonary vasoconstrictor fibres in the thoracic sympathetic nerves of the species.

With the methods used in the present experiments for measuring circulatory changes, interpretation of the observations was complicated by the effects of concomitant bronchomotor changes. None the less, a certain amount of valuable information has been obtained by analysis of a total of 311 blood outflow responses which occurred during as many stimulations of the stellate ganglia under a variety of experimental conditions and against a known background of intrapulmonary pressure changes. Of these observations, forty-six were obtained under control conditions, eighty-two after the addition of adrenaline only to the perfusion fluid and the remainder (183) either in eserinated preparations or in preparations treated with atropine or ergotoxine. In many experiments there were sufficient control observations with which to compare the responses obtained after injection of drugs.

In assessing these data, it was frequently found that, in response to stellate ganglion stimulation, the same order of bronchomotor change was not necessarily accompanied by an unvarying venous outflow response. On the other hand, the venous outflow responses might remain the same while the concomitant bronchomotor responses varied widely. Thus it appeared that the bronchial and vascular reactions to the same stimulus, i.e. the excitation of the nerves, were largely independent of each other. This conclusion was substantiated by a further analysis of individual experiments. The relevant facts and inferences about the pulmonary vasomotor responses may be summarized as follows:

Allowance having been made for possible mechanical vasoconstriction produced by a coincident rise in intrapulmonary pressure, it was possible to show that under certain conditions vasoconstriction occurred as the direct result of the nerve stimulations because (1) it was found frequently that with the same order of bronchoconstriction the reduction in blood outflow was at first very marked but subsequently became smaller until it disappeared altogether or remained only just perceptible; and (2) in

sixteen observations (out of a total of thirty-four), where no bronchomotor changes occurred owing to previous injections of drugs, it was observed that marked reductions in venous outflow occurred in response to stimulation of the stellate ganglion.

It was observed that vasodilatation occasionally occurred in response to stimulation of the stellate ganglia. For example, in one experiment a weak stimulus caused vasodilatation while a strong stimulus produced vasoconstriction. In others, vasodilatation was seen to occur after a series of gradually diminishing vasoconstrictor responses, an observation which suggests that vasodilator fibres came into action when the vasoconstrictor fibres were fatigued. With or without administration of drugs, the vasodilator response has been obtained in the absence of any bronchomotor change in eleven out of thirty-four observations. Individual experiments did not give any certain indication of the effects of adrenaline, atropine or ergotoxine on the vascular responses to sympathetic nerve impulses but when assessed in groups it was found that for a given order of bronchomotor change adrenaline did not alter the ratio of the total number of vasodilator to total number of vasoconstrictor responses (shown by comparison with the control groups); both ergotoxine and atropine doubled the percentage occurrence of vasodilator responses.

The foregoing evidence suggests that in the guinea-pig the lungs are innervated by sympathetic vasoconstrictor and -dilator fibres as well as by bronchoconstrictor and -dilator fibres.

(2) *Acetylcholine*

The effects of single injections of acetylcholine (1–100 μ g.) have been studied in twenty-eight experiments. Confirming the results of previous workers [Dale & Narayana, 1935; Petrovskaia, 1939; Thornton, 1939], it has been found that injections of acetylcholine into the pulmonary circulation cause bronchoconstriction.

The maximal bronchoconstrictor responses elicited by acetylcholine injections in the various experiments, and measured in terms of the positive change in intrapulmonary pressure (I.P.p.) varied for different doses of the drugs as shown in Table I.

Responses obtained in the adrenaline-Tyrode perfused lungs were uniformly less than those obtained in preparations in which either no adrenaline or small amounts (1–10 μ g.) had been added. This accounts for the discrepancy suggested by the values of the table, that the efficacy increases with doses from 1 to 20 μ g. and diminishes with doses greater than 20 μ g. The explanation is that the larger doses (25–100 μ g.) were

TABLE I

$\mu\text{g. ACh.}$	I.P.p. change + cm. H_2O	No. of experiments
1	0.8* to 3.8	4
2	0.8 to 10.9	3
5	1.0 to 18.8	3
10	0.6* to 24.4	13
15	15.6	1
20	0.6* to 25.6	6
25	3.8* to 5.0	2
30	2.0*	1
40	1.8*	1
100	5.0*	1

The figures with asterisks represent maximal responses obtained in experiments in which adrenaline had been added to the perfusion fluid ($1-4 \mu\text{g./c.c.}$). I.P.p. = intra-pulmonary pressure.

only used after the preparation had been rendered insensitive by adrenaline. Thus the depressant action of adrenaline on the activity of bronchoconstrictor nerves has an analogy in its effect on the bronchial reactions to acetylcholine. Although the actual mechanism of the suppression of these bronchomotor responses is not as yet clear, some of the conditions under which it occurs were demonstrated in experiments similar to the one shown in Fig. 7.

In twelve experiments both acetylcholine and stellate ganglion stimulations were tested under the same conditions and it was observed that the preparations most sensitive to acetylcholine were most sensitive to stimulations of the ganglia.

In an earlier communication [1939] I stated that the action of acetylcholine on the bronchi "is usually prolonged. Whether it is potentiated by eserine there is as yet no evidence on which to decide." This statement was made in reference to my own experiments only, as I should have made clear at the time, since it had already been shown by Thornton [1939] that in the guinea-pig both acetylcholine and vagal bronchoconstriction are potentiated by eserine (1 : 200,000). In recent experiments I have been able to confirm this result.

In the preliminary experiments when testing eserine potentiation of acetylcholine, only Tyrode-perfused preparations were used. With this type of perfusion a slight improvement of acetylcholine bronchoconstriction by means of eserine was observed in one experiment, while in another no effect on the response was observed. These more or less negative results were ascribed to the absence of esterase from the circulating fluid, a view supported by the prolonged acetylcholine-bronchoconstriction and the sluggishness of its recovery even in the non-eserinized preparation. Further support is given to this hypothesis by

more recent experiments on blood-perfused lungs in which recovery from acetylcholine bronchoconstriction was usually rapid and eserine potentia-

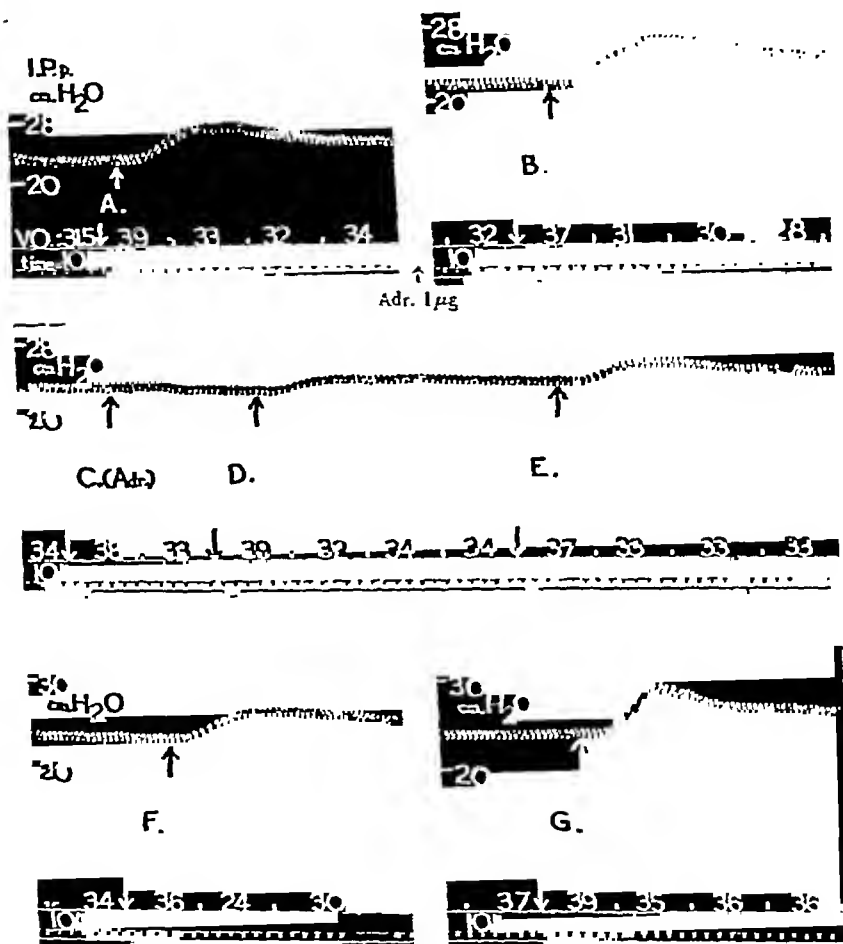


Fig. 7. 18. x. 39. Guinea-pig 85. \bar{z} ; 575 g. Pretreated with heparin (10 mg. intra-peritoneally) only. Perfused with blood-Tyrodé (1:1 dilution) in closed circulation at initial perfusion pressure of ~ 5 cm. perfusate. Tracings as in Fig. 1. At A (2.03 p.m.), B (2.25 p.m.), D (2.33 p.m.), E (2.37 p.m.), F (2.45 p.m.) and G (3.17 p.m.) injections of $10\mu\text{g.}$ of ACh. were given. Between A and B at 2.20 p.m. $1\mu\text{g.}$ of adrenaline was injected; and at C (2.31 p.m.) $10\mu\text{g.}$ of adrenaline was injected.

tion was readily demonstrated. In one case the potentiation amounted to about 100% and in another case (Fig. 8) it was about 450%. In the

experiment in Fig. 8, an approximately minimal effective dose of acetylcholine, $2\mu\text{g.}$, produced, in three observations, rises in the intrapulmonary pressure of $+0.4$, $+0.8$ (8A) and $+0.6$ cm. H_2O (8B), in that order. After the administration of eserine, the same amount of acetylcholine produced a pressure change of $+3.5$ cm. H_2O (8C). In both of the experiments quoted eserine increased not only the degree but also the duration of bronchoconstriction.

The finding of Dale & Narayana [1935] and of Petrovskaja [1939] that atropine abolishes acetylcholine bronchoconstriction has been confirmed in three experiments. The dose of atropine required to produce this effect varied from 100 to $300\mu\text{g.}$ where the total perfusion volume was about 20 c.c. (recirculated). Suppression by atropine of the bronchomotor

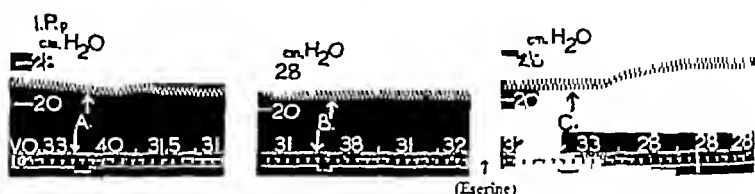


Fig. 8. 1. xi. 39. Guinea-pig 88. ♂; 390 g. Pretreated with heparin (10 mg. intraperitoneally) only. Perfused with blood-Tyrode (1:1 dilution) in closed circulation at initial perfusion pressure of $+5$ cm. perfusate. Tracings as in Fig. 1. At A (3.12 p.m.), B (3.18 p.m.) and C (3.42 p.m.), injections of $2\mu\text{g.}$ of ACh. were given. Between B and C at 3.22 p.m. $100\mu\text{g.}$ of eserine were added to the reservoir.

response to stellate ganglion stimulation and acetylcholine was obtained in one experiment in which ergotoxine also suppressed these responses (Fig. 3).

In nicotinized preparations, the bronchial response to acetylcholine was much the same as in the untreated lungs (eight experiments). Compared with control observations the acetylcholine bronchoconstrictor effect was of the same order after nicotine had been injected. The effect of nicotine on the pulmonary responses to acetylcholine and the stellate ganglion is shown in the experiment to which reference has already been made (Fig. 6).

The contraction of the bronchial smooth muscle to acetylcholine, the potentiation of this effect by eserine, and its suppression by atropine were phenomena to be expected in the light of our present knowledge of acetylcholine action, but the reduction or suppression of acetylcholine effects by ergotoxine have been reported only recently [Foggie, 1940].

The results of twenty-four experiments showed that in suitable concentration ergotoxine suppressed acetylcholine bronchoconstriction (see Fig. 3). Recovery of the response from ergotoxine occasionally occurred within 30 min. but was usually more delayed (1-2 hr.). The effective dose of ergotoxine depended partly upon the amount of acetylcholine being given by single injection and partly on circumstances discussed more fully below.

Injection of ergotoxine itself often increased the intrapulmonary pressure level so that a possible explanation of the reduction of the acetylcholine response by ergotoxine was that the latter drug may have increased the tonus of the bronchi so that they were incapable of further contraction. This explanation has been rendered invalid by the observation that after suppression by ergotoxine, the bronchoconstrictor response may recover with repeated injections of acetylcholine, although the intrapulmonary pressure level between injections remains approximately the same. Also it has been found that suppression of the response may continue even when the level has fallen considerably. Finally, in several experiments in which the intrapulmonary pressure was the same after ergotoxine as before, suppression of the acetylcholine response nevertheless occurred.

The doses of ergotoxine varied from 10 μ g. to 1.5 mg., which correspond to perfusion concentrations of 1:2,500,000 to 1:1300. The more usual concentrations employed were from 1:25,000 to 1:50,000. The effectiveness of any given dose depended chiefly upon the sensitivity to acetylcholine originally exhibited by the individual lung preparations. It was also seen that while a given amount of ergotoxine might abolish the bronchoconstriction produced by a given amount of acetylcholine, it did not necessarily suppress the effect of larger doses of acetylcholine. The effective suppressive dose of ergotoxine varied between 300 μ g. and 1.0 mg. when the dose of acetylcholine was 10-20 μ g. In a few tests only 1 mg. of ergotoxine was insufficient to suppress the response entirely although it produced a reduction of 50%.

It was observed that in adrenaline-Tyrode perfused preparations in which the bronchoconstrictor responses to acetylcholine were less than in adrenaline-free preparations, relatively small doses of ergotoxine (300 μ g.) abolished these responses entirely. However, the action of ergotoxine in suppressing acetylcholine bronchoconstriction was not dependent upon the addition of adrenaline to the pulmonary circulation, since suppression was produced in preparations to which no adrenaline had been previously given either before or after the death of the animal.

From these and other control experiments it became evident that adrenaline and ergotoxine may act synergistically to abolish acetylcholine bronchoconstriction but they can each produce the effect independently of one another.

The effect of ergotoxine on acetylcholine bronchoconstriction was the same in nicotinized preparations as in the untreated lungs (four experiments).

With regard to the pulmonary vascular responses to administration of acetylcholine, little can be added to the results of Petrovskaja [1939] and those of Dale & Narayana [1935]. Like the former author, I have found that the most usual effect of acetylcholine injection was bronchoconstriction associated with a diminished venous outflow. The results of the control observations can be expressed as follows:

- I.P.p. + associated with V.O. - in 23 experiments,
- I.P.p. + associated with V.O. + in 1 experiment,
- I.P.p. + associated with V.O. + and - in 1 experiment,
- I.P.p. + associated with V.O. change doubtful in 3 experiments,

where I.P.p. + = a rise in intrapulmonary pressure and V.O. + and - = increase and decrease respectively in the venous outflow.

The evidence obtained was not sufficient to decide whether the reduction in venous outflow observed in the majority of experiments was due to the vasoconstrictor action of acetylcholine or was a mechanical effect consequent upon the coincident bronchomotor response. Individual observations indicated that acetylcholine may have acted directly on the pulmonary blood vessels to produce constriction, because the venous outflow reduction was often much greater than would be expected, if it were solely due to the bronchomotor change accompanying it; and because very often in the same experiment the venous outflow responses to acetylcholine injections varied, while the bronchomotor responses remained fairly constant and vice versa.

It has been reported by Dale & Narayana [1935] that perfusion of guinea-pig lungs with adrenaline in a concentration of 1 : 250,000 intensifies the normal vasoconstrictor reaction to acetylcholine. Petrovskaja [1939] was unable to find that adrenaline produced such an effect when given in small single injections (1-2 μ g.). In this connexion it may be observed in the experiment shown in Fig. 7 that whereas an initial injection of 10 μ g. of acetylcholine did not reduce the pulmonary venous outflow rate at all, a second injection a few minutes subsequent to the administration of 1 μ g. of adrenaline did do so to a noticeable degree. The

effect of $10\mu\text{g.}$ of adrenaline was not so definite. In view of the fact, however, that with a dose of $1\mu\text{g.}$ the concentration of adrenaline in the pulmonary circulation would be equivalent to the perfusion concentration used by Dale and Narayana, the experiment is interesting as a confirmation of their results.

It should be added that acetylcholine injections have been observed to produce vasoconstriction in the nicotinized as well as in the normal perfused guinea-pig lungs (cf. Fig. 6). Also, as might be expected, the vasoconstrictor response to acetylcholine tended to disappear when atropine or ergotoxine had been added to the perfusate (cf. Fig. 4). Whether or not these effects are attributable to a direct action on the pulmonary blood vessels or are an expression of concomitant bronchial responses cannot be decided on the evidence of the experiments discussed here.

DISCUSSION

The most interesting finding of the experiments which have been described is the observation that the administration of acetylcholine and the excitation of the upper thoracic sympathetic nerves both produce bronchoconstriction in the guinea-pig. A second point is that, so far as the investigation has proceeded, the same conditions have been found to govern the response to each of these two stimuli. In both cases the bronchoconstrictor response is enhanced by eserine, temporarily depressed by adrenaline, and wholly or partly suppressed for a longer period by atropine or ergotoxine. The parallel suggests that the bronchoconstrictor fibres excited by stimulation of the stellate ganglion may be cholinergic. It may appear at first sight somewhat paradoxical that the suppression by ergotoxine of stellate ganglion bronchoconstriction is brought forward as evidence that the response involves the participation of cholinergic nerve fibres, yet the fact that ergotoxine suppresses the bronchoconstrictor activity of acetylcholine is sufficient justification for tentatively adopting this line of reasoning.

No explanation has as yet been found to account for the action of ergotoxine in suppressing acetylcholine bronchoconstriction. Ergotoxine was originally used for the purpose of determining whether or not the sympathetic bronchoconstrictor fibres were adrenergic. It is worth noting in this connexion that Petrovskaja [1939] has suggested that among the cervical vagal and cervical sympathetic fibres there may be some adrenergic bronchoconstrictor fibres, since the bronchoconstrictor effect of cervical vago-sympathetic stimulation may be suppressed or reversed by ergotoxine. My own subsequent experiments revealed, how-

ever, that ergotoxine suppresses the acetylcholine bronchial response as well, so that there are no longer grounds for assuming that ergotoxine acts specifically to prevent only adrenergic nerve motor responses. Nor can it be assumed that ergotoxine will not act to prevent the motor response to cholinergic nerve stimulation.

That these conclusions may have a more general application is suggested first by experiments reported by Foggie [1940], in which she found that ergotoxine and adrenaline reverse the pulmonary vasomotor effect of acetylcholine in the nicotinized perfused lungs of the dog; and secondly by Petrovskaja's observation that vagal bronchoconstriction in the guinea-pig is reversed by ergotoxine; and finally by the experimental results of Brown, McSwiney & Wadge [1930], who have reported that ergotoxine may suppress the motor response of the gastric musculature to stimulation of the sympathetic nerve supply.

The experimental evidence reviewed here appears more surprising in view of the results of Matthes [1930] and of Loewi & Navratil [1926] showing that ergotamine and ergotoxine may enhance the action of acetylcholine by inhibiting the action of esterase. No effect comparable to this was noted either by Petrovskaja or by me in the study of the isolated perfused guinea-pig lungs.

A recent report by Linegar [1940] on the related actions of acetylcholine and ergotamine should also be mentioned here. This worker found that in dogs and cats treated with atropine or with eserine and atropine, ergotamine reverses the pressor action of acetylcholine; and he concludes that ergotamine accomplishes the reversal by increasing the sensitivity of the blood vessels to the vasodilator action of acetylcholine. In so far as the present experiments are concerned, there is as yet no evidence for believing that acetylcholine exerts any dilator action on the bronchi, so that it is unlikely that suppression of the acetylcholine bronchoconstrictor response by ergotoxine depends upon the mechanisms suggested by Linegar [1940] for the systemic blood vessels.

SUMMARY

1. The most usual effect of electrical stimulation of the stellate ganglion on the isolated perfused lungs of the guinea-pig is the production of marked bronchoconstriction. Occasionally, under special conditions, a dilator response also occurs.

2. The bronchoconstrictor response to stellate ganglion stimulation is qualitatively similar to that produced by injection of acetylcholine. Both are potentiated by eserine, temporarily depressed by suitable doses

of adrenaline, and are suppressed wholly or partly by ergotoxine and by atropine.

3. The bronchoconstriction following stimulation of the stellate ganglion or injections of acetylcholine occurs in nicotinized lung preparations as well as in the normal preparations.

4. Some evidence has been given that in addition to bronchoconstrictor and dilator fibres there are vasoconstrictor and dilator fibres reaching the lungs by way of the stellate ganglion.

5. The evidence suggests that acetylcholine may act directly on the pulmonary blood vessels to produce vasoconstriction as distinct from mechanical effects exerted by the coincident changes in the bronchi.

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OBSERVATIONS ON THE FUNCTION
OF THE ROUND WINDOWBy C. S. HALLPIKE¹ AND P. SCOTT²*From the Ferens Institute of Otology, Middlesex Hospital,
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ACCORDING to the resonance hypothesis the mechanical system of the cochlea is held to comprise an assembly of units, each resembling an acoustic receiver of the so-called displacement type, each operating at resonance and of correspondingly high efficiency. It is further held that the efficient working of this mechanism, at any rate for low frequencies, is dependent upon the freedom of movement of the round window membrane. The physical basis of this view is provided in part by the great length of low-frequency sound waves in fluid relative to the dimensions of the cochlea itself, and in part by the rigidity of the bony capsule of the labyrinth. Thus at 50 c./sec., the wave-length in water is 24 m., whereas in the human subject the length of the fluid column between the oval and round windows via the helicotrema is only 60 mm. The difference in phase between the two windows is thus rather less than 1°. It follows that movement of the windows and the intervening fluid is virtually a mass movement, and that obstruction of the round window must bring about obstruction of the entire system. This argument is dependent in almost equal measure upon the generally made and reasonable assumption that the relative rigidity of the bony labyrinth wall makes it necessary to regard the round window as the only significant low-resistance pathway.

Animal experiments and an observation upon human pathological material to be described in the present paper now show that these considerations are unrelated to the physiological mechanism of hearing.

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1. THE PHYSIOLOGICAL EFFECT OF EXPERIMENTAL OCCLUSION OF THE ROUND WINDOW IN THE CAT

Methods

Under light chloralose anaesthesia the round window niche of the cat can readily be exposed by dissection through the neck. The preparation was arranged with a brass tube tied into the external auditory meatus and with suitable recording electrodes in the contra-lateral auditory tract. Acoustic stimulation was then applied at 100 and 150 c./sec. and the action potentials observed and recorded.

Full details of the technical equipment and methods employed are given in a previous publication [Hallpike & Rawdon-Smith, 1934]. The intensity of the stimulus was so adjusted that small intensity changes (2 db.) were clearly reflected in the response, as measured upon the face of the cathode-ray tube. Derbyshire & Davis [1935] have described the sigmoid form of the curve which expresses the relationship of stimulus intensity to the voltage of the resulting auditory action potentials. The present measurements would of course coincide with the most vertical portion of this curve, corresponding to some 55 db. above the human threshold.

The round window niche was then filled with warm Ringer's fluid with careful exclusion of air bubbles. Plaster of Paris powder was then added gradually and mixed *in situ* with a small ball-pointed probe. When the setting of the plaster had begun a small glass disk was laid over the niche and pressed down into firm contact with its margin with a glass rod, in this way squeezing out all excess of soft plaster.

Setting of the plaster was well advanced in 2 or 3 min., a period over which it has been found possible to rely upon the stability of the tract response.

Following the setting of the plaster the sound stimulus was again applied at its initial intensity and the tract response once again measured. All observations were made by direct measurement upon the face of the cathode-ray tube.

RESULTS

Observations upon these lines were carried out upon fourteen cats, and in all the tract response following occlusion of the round window niche was found to be unaltered.

2. THE EFFECT UPON HEARING IN A HUMAN SUBJECT OF BONY OCCLUSION OF THE ROUND WINDOW

Clinical history

A patient, under the care of Dr J. Purdon Martin, died at the National Hospital, Queen Square, on 14 September 1938, and post-mortem examination revealed a glioma of the left side of the pons. The patient had suffered from tinnitus and deafness in the left ear. The hearing was examined one week before death. There was total deafness of the left ear. In the right ear, however, the watch and whispered voice were heard at the normal distance, and all of the Bezold-Edelmann series of tuning forks were heard down to and including 16 c./sec. It must be regretted that an audiometer was not used for testing the hearing for the higher tones. Nevertheless, the Bezold-Edelmann forks still provide what is probably the best test for low-frequency deafness. In particular, the maximum output of the lowest forks is little, if any, above the normal human threshold, and a positive response to the 16 c./sec. fork can therefore be regarded as very good evidence of an unimpaired mechanical condition of the peripheral auditory apparatus.

Histological examination

The temporal bones were removed 14 hr. after death, fixed in 4% formaldehyde and embedded in celloidin. Serial sections were cut in the vertical plane at 25μ . In the right labyrinth there was found a focus of otosclerosis in the usual position adjoining the oval window without, however, any involvement of the stapes footplate. In the scala tympani a mass of otosclerotic new bone was discovered which appeared in large part to occlude the round window from within.

The condition is shown in the photomicrograph (Fig. 1). In places the occlusion was incomplete, although where this was the case the membrane was found to be replaced by dense fibrous tissue. The region of the round window was further studied more precisely by anatomical reconstruction and the full extent of the bony occlusion was measured.

The results showed the area of the membrane unoccluded by bone to be 0.5 mm.^2 . The area of the membrane in the opposite temporal bone was 1.7 mm.^2 , representing a reduction on the affected side of some 70%. The cochlear aqueduct was patent. In the left temporal bone foci of otosclerosis were also found in the neighbourhood of the round and oval windows, but with no occlusion of either.

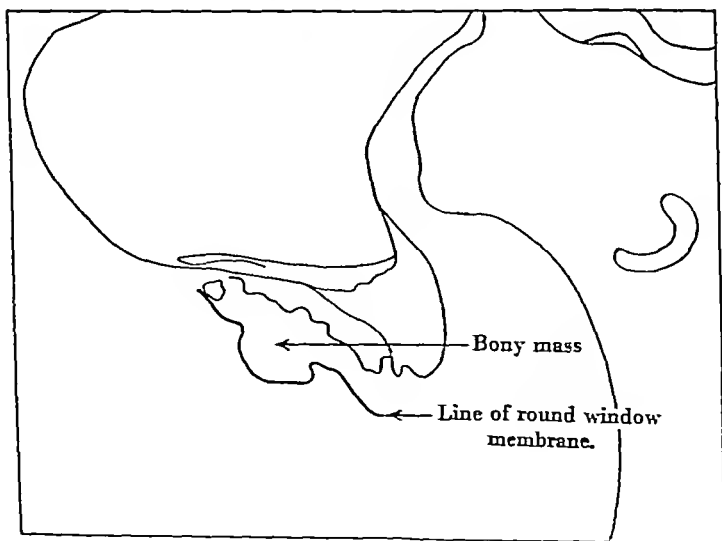


Fig. 1.

which might well reproduce the physical constants of the living labyrinth capsule the value of r is given as 22×10^4 . Thus a likely value for η at the fluid/bone interface in the ear would be 0.95.

Nevertheless, it seems clear that the formula given is computed for the case of extended media, and its application to the confined spaces of the labyrinth must be regarded as open to considerable question.

A further and considerable difficulty in assuming any free transmission of sound through the bony labyrinth wall lies in the extreme attenuation of sound, air conducted to one cochlea, in its transmission through the skull to the opposite cochlea. The figure given by Fletcher [1923] for this attenuation is 60 db.

According to Pohlman's hypothesis, the first cochlea might be regarded as a point source transmitting to a point receiver in the opposite cochlea. In these circumstances, the resulting attenuation would follow the law of inverse squares. If the source be taken as the stapes footplate, then the approximate distances to the nearest points of the cochlear spirals in the same and opposite ears are 2.8 and 80 mm. respectively giving an extreme attenuation of some 30 db. For these reasons it would appear to be impossible to accept Pohlman's hypothesis.

Other data on the effects of experimental occlusion of the round window have been provided by the extensive experiments of Hughson & Crowe who have reported in detail [1932*a, b*] the results of a large series of experiments in which the round window niche of the cat was lightly packed with moist cotton wool or connective tissue grafts. The effect of this procedure was then observed upon the mixture of cochlear and auditory nerve action potentials which can be recorded from an uninsulated grid electrode introduced through a small opening in the skull and located close to the trunk of the VIII nerve, with an indifferent earthed electrode in the neck muscles.

A marked increase of this electrical response was observed following the occlusion of the round window niche and was considered to connote an increase of cochlear sensitivity.

Certain difficulties arise, however, in connexion with this interpretation. The degree of immobilization of the round window membrane obtainable with cotton wool may well be insignificant. In addition, certain possibilities of electrical artefact do not appear to have been fully excluded. If the cochlear potentials are recorded from an electrode upon the mucous membrane of the promontory a similar change in amplitude occurs on plugging the niche of the round window with a moist cotton-wool plug as Hughson & Crowe described.

This, however, seems very likely to be an electrical artefact. The main electrical pathway from the interior of the cochlea is through the round window on to the surrounding mucous membrane, and the effect of a moist plug of cotton wool in this situation would be to increase the electrical response from an electrode more distantly placed by bringing it electrically nearer to the source of the potential changes, an explanation which may possibly be applicable under the experimental conditions which Hughson & Crowe employed.

As already indicated, the present findings are not explicable upon the conventional type of displacement hypothesis which involves freedom of movement of the fluid columns bounded by the oval and round windows, or upon Pohlman's hypothesis of a free transmission of acoustic energy through the bony wall of the labyrinth.

As an alternative explanation it can be suggested that the pressure-sensitive cochlear receptors are suspended in a fluid-containing duct of high internal resistance. This resistance is dependent mainly upon high frictional losses and is of such a magnitude as to render insignificant any alterations in the resistance of the round window. Since the damping in such a system would seem likely to be at least critical, it must be agreed that such a hypothesis is clearly opposed to the idea of mechanical resonance in the cochlea. Nevertheless, the animal experiments of Witmaack [1907] and of Yoshii [1909] and the observations on human pathological material by Crowe, Guild & Polvogt [1934] as well as much evidence from other sources remain to indicate the occurrence of frequency discrimination within the cochlea of the traditional pattern. Whether these findings will prove to depend upon factors intrinsic in the sensory cells and their neurones or upon mechanical factors of the type envisaged by Reboul [1938] cannot at present be decided.

With regard to some positive explanation of the function of the round window, Pohlman and others have already suggested that its membrane operates as a pressure-stabilizing device in response to slow displacements of the stapes footplate. Alternatively, the possibility of its connexion with the flow of the perilymph appears to be well worthy of consideration. As is well known, the endolymph sac lies within the bony walls of the labyrinth in a bath of fluid, the perilymph, which reaches the perilymph spaces from the sub-arachnoid space of the posterior fossa by a narrow bony channel, the aqueduct of the cochlea. It follows that in the absence of a distensible vent in the bony labyrinth wall such as the round window membrane the bulk modulus of the labyrinth cavity would attain very high proportions approximating to that of water itself, and the circulation

of cerebro-spinal fluid through the cochlear aqueduct in response to pressure changes in the subarachnoid space would thus be brought to a standstill.

Although the physiological importance of such a circulation has not yet been defined, it does in this way appear possible to assign to the round window membrane an alternative to its more traditional acoustic function.

SUMMARY

No change of cochlear sensitivity to tones of low frequency has been found to result from

(a) Experimental occlusion of the round window membrane in the cat.

(b) Occlusion of the round window membrane in a human subject by a pathological formation of new bone.

These findings are thought to contra-indicate an acoustic function of this membrane as postulated by the resonance hypothesis. Certain alternative explanations are considered.

Our thanks are due to Drs J. Purdon Martin and J. G. Greenfield of the National Hospital, Queen Square, for making possible the clinical and pathological observations described in this paper, and to the Medical Research Council for the provision of an assistant to one of us (C. S. H.). The expenses of the histological work were borne in part by a grant from the Gamble Research Fund of the Royal Society of Medicine.

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THE AUTOMATIC COLLECTION OF LUNG GASES IN CATS

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THE value of a method which obtains samples of alveolar air in cats lies mainly in the fact that the study of respiratory problems in these animals is severely handicapped without a knowledge of the tensions of the gases in the lungs.

The apparatus

The apparatus consists of a bivalve "mouthpiece" modelled on that used by Henderson & Haggard [1925] for human subjects (see Fig. 1). Connected with this by two small tubes *A* and *B* is a circuit composed of two sampling tubes *S.T.*₁ and *S.T.*₂, a blood gas analysis bottle *G.A.B.* and a Muller valve *M.V.* The valves of the mouthpiece were made of mica flaps supported by phosphor bronze springs. The valve is packed with plasticine to reduce the dead space.

PROCEDURE

The tube *T* is tied into the lowest part of the trachea in the neck. When the cat expires it will do so through the valve *V*₂, and the last portion of the expired gases will lie around the proximal part of the expiratory tube *E.T.* During inspiration the air is sucked through the valve *V*₁. At the same time a small amount of air is sucked through the tube *A*, which will cause the gases to be circulated through the system of sampling tubes, etc., so that a small sample of the end of the previous expiration is drawn through the tube *B*. Eventually the sampling tubes and the bottle *G.A.B.* will fill with gases from the lungs of the cat.

A series of samples was collected from decerebrated and chloralosed cats. The volume of the sample that was withdrawn with each inspiration was approximately 2.5 c.c.; reduction of this to 0.7 c.c. caused no alteration in the value of the CO₂ tension. The time allowed for the first sampling tube to fill was 10 min.; longer periods did not produce any change in the percentage of the gases.

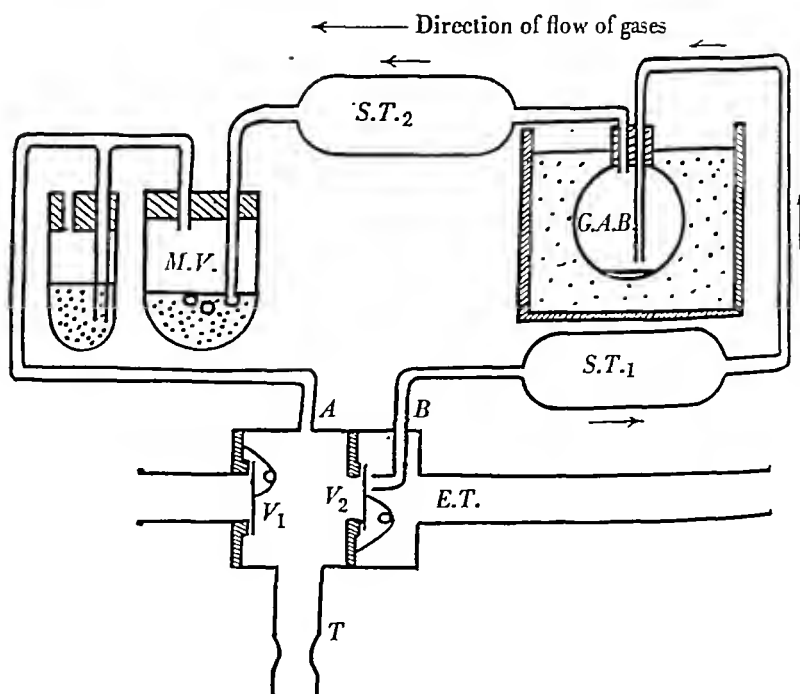


Fig. 1.

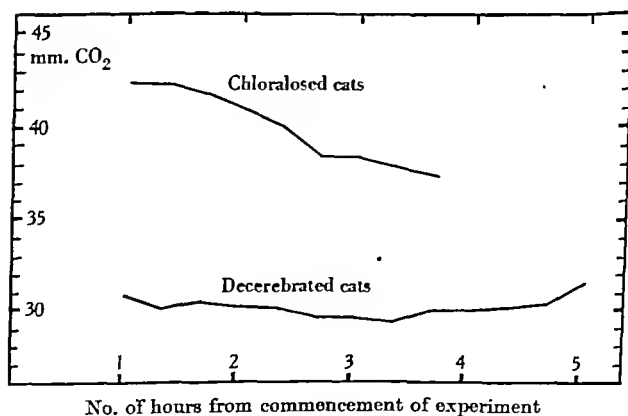


Fig. 2.

The results of four experiments on decerebrate cats were graphed, and the average of the figures obtained is given in Fig. 2. In Table I are the results from a single experiment.

TABLE I

Time	CO ₂ % in samples mm.	Tidal vol. c.c.	No. of respirations per min.
(Noon. Decerebration completed)			
1.10	30.0	41.7	15
1.47	30.3	41.7	14
2.24	30.8	40.0	18
2.58	29.7	31.3	23
3.35	29.2	39.4	25
4.10	30.7	65.7	9
4.47	33.5	—	7
5.15	33.3	—	—
5.35 Cat died			

The results from five experiments under chloralose (0.07–0.08 g./kg.) were similarly graphed. The results are also presented in Fig. 2. The results from one experiment are given in Table II.

TABLE II. Chloralose 0.07 g./kg. injected noon

Time	CO ₂ % in samples mm.	Tidal vol. c.c.	No. of respirations per min.
12.45	48.3	21.7	16
1.23	46.7	23.2	16
1.42	44.9	24.4	18
3.24	40.4	25.0	20
3.51	39.0	25.0	17

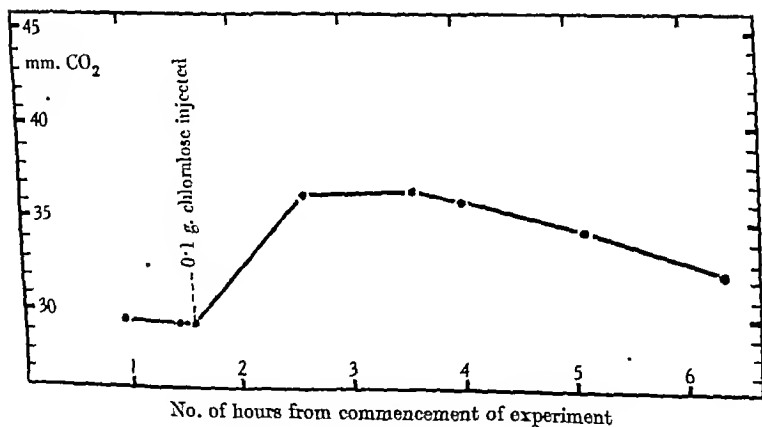


Fig. 3.

The results of these experiments show that chloralose gives results which are higher than those of decerebrate animals. The writer suggests that the level of the CO₂ tension in the lung gases, when caused to rise by

the chloralose, is an indication of the depth and duration of the depression of the respiratory mechanism. Such an effect is shown in the results of one experiment in Table III and Fig. 3. On this occasion the cat was first decerebrated, and the partial pressure of the CO_2 in the lung gases was estimated before and after the injection of 0.1 g. of chloralose. The effect of repeated injections of Evipan sodium and chloralose are also shown in Table III.

TABLE III (see Fig. 3)

Time		Time	
10.30	Decerebration completed	2.30	Decerebration completed
11.25	29.6 mm. CO_2	3.15	24.8 mm. CO_2
11.55	29.4	3.30	24.6
12.05	0.1 g. chloralose injected	3.32	0.03 g. Evipan injected
1.05	36.3	4.00	28.7
2.05	36.6	4.30	26.2
2.30	36.1	4.45	25.6
3.35	34.5	4.50	0.02 g. Evipan injected
4.40	32.3	5.10	26.5
		5.30	25.2
		5.45	25.7
		5.46	0.08 g. chloralose
		6.10	30.8
		6.25	27.9

THE COMPARISON OF THE TENSIONS OF THE GASES IN THE SAMPLES OF LUNG GASES AND THE ARTERIAL BLOOD

Cats were anaesthetized with chloralose (0.07–0.08 g./kg.) and heparin was injected. A cannula which was of sufficient length to reach the aortic arch was inserted into the left carotid. A sample of blood was withdrawn and placed in the bottle *G.A.B.*, in which there was a trace of potassium oxalate. The lung gases were allowed to flow through the circuit as described above. The equilibration of the blood with the gases was assisted by shaking the bottle with a motor. The bottle *G.A.B.* was immersed in a water-bath at the cat's body temperature. It was important to know how long it took to fill the gas analysis bottle with the lung gases. This was carried out by determining the period required to fill the tubes *S.T.*₁ and *S.T.*₂ with the same percentage of CO_2 . This occurred in 20 min. The volume of each sampling tube was 25 c.c. and the bottle 40 c.c.

After the equilibration period had elapsed the blood was covered with a layer of paraffin, and at the same time a sample of blood was withdrawn from the carotid cannula. The CO_2 content of these samples was estimated. The difference between the CO_2 content of the arterial and equilibrated blood in seven cats is shown in Table IV. The average of these figures shows a difference of 0.9 vol. % CO_2 . The results when

extrapolated on an average dissociation curve for cat's blood show a tension difference of approximately 1.75 mm. Such a difference cannot be regarded as significant.

TABLE IV

Exp.	Time	Difference between the CO ₂ content of arterial and equilibrated blood
1	Noon	Operation finished
	12.32	0.4 vol. %
	1.22	1.7 vol. %
2	9.45	Operation finished
	11.25	0.4 vol. %
	12.15	2.0 vol. %
3	10.00	Operation finished
	10.40	0.6 vol. %
	11.40	0.4 vol. %
4	10.20	Operation finished
	11.03	4.4 vol. %
5	10.00	Operation finished
	10.35	0.8 vol. %
6	10.00	Operation finished
	12.10	2.8 vol. %
7	10.20	Operation finished
	10.45	-0.3 vol. %
	12.00	-3.3 vol. %

Average = 0.9 vol. %.

Tension difference = 1.75 mm. Hg.

DISCUSSION

The use of fractional methods in human subjects has been criticized mainly on the grounds that those who have a small tidal air may not wash out their dead space, and samples of alveolar air will not be obtained. In the above experiments the dead space of the animal is largely abolished by inserting the "mouthpiece", the dead space of which was 2.25 c.c., into the trachea. Smith & Heinbecker [1928] applied a fractional method to dogs. They used a mask similar to that brought out by Marshall [1926], which is fixed on the dog's nose. They compare the tensions in the samples with those of the blood obtained by left ventricular puncture. Their results when averaged show that the blood samples were lower than the lung gas samples by 0.22 mm. Hg. They concluded that the blood and the alveolar air were in equilibrium as regards CO₂ within the limits of error. The work on rabbits by Krogh & Krogh [1910] produced a difference of 3 mm. Hg, the blood tensions in this latter instance being higher than the air of the "tracheal bifurcation". It might be argued, about the experiments performed here, that with the steady fall in alveolar CO₂ in the chloralosed cats the CO₂ in the equilibra-

tion bottle might have a higher pressure than the true alveolar air at that moment, and thus diminish the difference between the arterial and equilibrated samples. If such were the case the percentage of CO_2 in the tube $S.T._1$ would be higher than that in $S.T._2$. This was shown not to be the case. The tensions were the same after 20 min. or longer. Again when samples were taken prior to equilibration (two samples) or during equilibration (one sample) the differences observed were within the limits of experimental error.

SUMMARY

The automatic collection of lung gases in cats is described and the depressent effect of chloralose on the respiratory mechanism is demonstrated by the alterations it produces in the partial pressure of the CO_2 in the lungs.

Thanks are due to Mr F. S. Wilson for making the "mouthpiece".

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THE INDIVIDUAL AND INTEGRATED ACTIVITY OF THE SEMICIRCULAR CANALS OF THE ELASMOBRANCH LABYRINTH

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(Received 8 June 1940)

THE recent work of Steinhausen [1931, 1935] has furnished direct experimental confirmation of the classical theory of the mechanism of the semicircular canals, inasmuch as it was clearly shown that the cupula terminalis, the gelatinous auxiliary structure enclosing the sensory hairs of the crista ampullaris, is deflected from its normal position during an appropriate angular displacement of the labyrinth. At the same time, Steinhausen was able to demonstrate and analyse the correlations between the deformation of the cupula and the occurrence of reflex eye movements. Our own electrical study of the horizontal canal of the dogfish [Löwenstein & Sand, 1936] disclosed the unexpected fact that the sensory excitation which, on the grounds of reflex phenomena, could be presumed to occur during ipsilateral rotation, manifested itself as an increase in the volume of a persistent impulse discharge which occurs spontaneously in the stationary labyrinth, and that, further, this spontaneous discharge was reduced, or even completely inhibited, during a rotation in the direction contralateral with respect to the canal under investigation. It appears, therefore, that the sensory mechanism of the ampulla is such that rotation in the plane of the canal in either direction evokes a characteristic sensory response, and since it is certain that the increased excitation of the ampulla during ipsilateral rotation is the causal agent in the release of responses such as reflex eye movements, it may be surmised that the inhibition which occurs at the same time in the corresponding ampulla on the other side of the head may also play a part in the central nervous processes which determine the pattern of reflex motor discharges [Löwenstein, 1937].

In order to gain some insight into this problem it is clearly desirable to extend the observations to include all the semicircular canals, so that one may be in a position to specify the events which occur in the sensory connexions of all six ampullae during an angular displacement of the head in any given plane. This we have attempted to do, and the results are the subject of the present communication.

METHODS

Electrophysiological analysis of the activity of the separate parts of the vertebrate ear has progressed slowly because of the extreme inaccessibility of these structures. Our own experiments on the horizontal canal of the dogfish (*Scyllium canicula*) were the first attempt to record the sensory discharges from an individual ampulla. The ampullae of the other two semicircular canals, the anterior vertical and posterior vertical, are even less accessible, and the task of obtaining records from the nerve twigs supplying them appeared at first sight almost hopeless. The successful experiments of Ross [1936], who recorded action potentials from the isolated labyrinth of the frog, and the experience of one of us [Sand, 1938] with isolated elasmobranch sense organs encouraged us to explore the possibility of working with the isolated labyrinth, in which the exposure of individual ampullae and their nerves can be accomplished with a certainty and neatness which would be impossible in the whole animal. The attempt was successful; the isolated labyrinth remains functionally active for several hours, and it was possible to obtain satisfactory oscillographic records from all three semicircular canals.

Mature specimens of the thornback ray, *Raja clavata*, were used, having a wing span of 18–20 in. The fish is killed by pithing, the entire jaw apparatus is quickly removed, the eye is excised, and by a median longitudinal cut through the head one-half of the cranium is separated and pinned in a convenient position by the remaining strip of rostrum to a board. The jaw muscles and eye muscles attached to the cranium are removed, and the cartilaginous auditory capsule is now cleanly exposed for dissection. The posterior ampulla, which lies at the back of the labyrinth, is approached from the floor of the auditory capsule, i.e. from the posterior region of the palate. The anterior and horizontal ampullae are approached from the outer anterior surface of the capsule, i.e. from the posterior region of the orbit. When a little cartilage in the appropriate region has been sliced away, and before the cavity of the labyrinth is actually opened, the ampulla and its nerve become clearly visible, and one can then proceed carefully shaving away a groove of cartilage sufficient to allow the nerve to be exposed, ligated, cut centrally, and lifted at its free end, its attachment to the ampulla remaining, of course, intact. Thus only a small opening is made into the perilymphatic cavity, and very little perilymph escapes. The membranous labyrinth itself must not be perforated, and this is not difficult to avoid owing to the excellent visibility of the preparation. Should the sacculus or utricle be perforated, the preparation is spoiled, and this is soon evidenced by its behaviour when installed for electrical recording

It is advisable to use a dissecting microscope for the later stages of the preparation, though the whole process can be carried out with the naked eye. About 4 mm. of nerve is available from the posterior ampulla, and 2 or 3 mm. from the anterior and horizontal ampullae.

When a nerve from one of the ampullae has been prepared, the cartilaginous capsule containing the labyrinth is severed from the remaining anterior portion of the cranium and rostrum which has served for its fixation during the dissection, and is mounted in one of the two electrode-holders illustrated in Figs. 1 and 2.

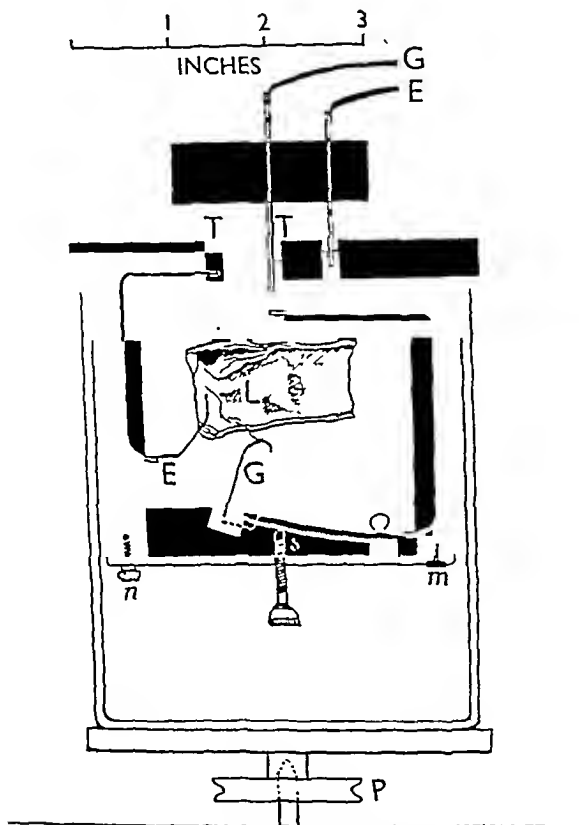


Fig. 1. Diagram of rotating holder. Explanation in text.

The rotating holder (Fig. 1) is a circular slab of ebonite turned to fit accurately, like a lid, into a cylindrical glass jar. Two pillars set opposite each other on the under side of the slab are joined by a brass strip which swings aside about the pivot *m* and can be secured by a screw *n*. In the centre of the slab is a well of circular cross-section, and concentric with it is an annular trough less deep than the well. The well and the annular trough *T, T*, are filled with mercury, and into each dips a stout copper rod. The two rods are held in a block of ebonite, which is clamped in an adjustable stand so that it may be raised and lowered. The input leads to the amplifier are soldered to these two copper rods. The ebonite slab and pillars are drilled to take the two copper wires which connect the mercury in the trough

T, T with the platinum electrodes G, E . The grid electrode G is given a mechanical vertical adjustment by being mounted on the spring s , attached at one end to the brass strip nm . The glass jar is glued in an accurately centred position to the turn-table, which is driven by means of its pulley, P .

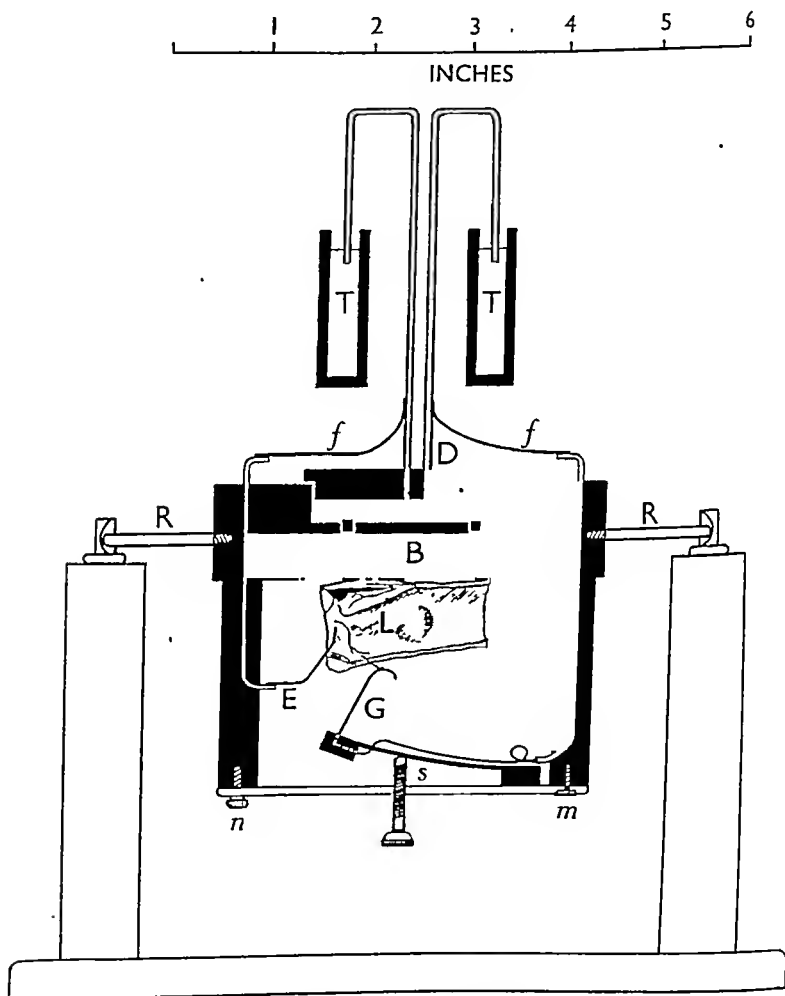


Fig. 2. Diagram of tilting holder. Explanation in text.

For mounting a preparation the ebonite holder is removed and laid upside down on the bench. The labyrinth L is then secured firmly in position by means of three rubber bands which cross over it and are fastened to six screw hooks set peripherally around the under surface of the slab. The brass strip nm is swung into position, and the nerve adjusted on the electrode G . The neutral electrode E is brought into contact with the body of the preparation. The holder is then turned the right way up and lowered into position in the jar on the

turn-table. Mercury is deposited in the troughs, the copper rods are brought down to make contact with it, and the installation is now ready for recording. The mercury has to be emptied and renewed every time a fresh preparation is mounted. It is a convenient circumstance that the upper surface of the head in a ray, above the auditory capsules, is flat and horizontal. Therefore, when a labyrinth is mounted as described, its orientation corresponds fairly accurately with its normal position in space in a ray lying flat on a horizontal surface.

The tilting holder (Fig. 2) is similar in design except that the ebonite slab *B* is tapped to take four brass rods, *R, R*, at intervals of 90° around its circumference. These serve to pivot the holder for tilting the labyrinth about its longitudinal or transverse axis. Connection with the amplifier is made through two long parallel mercury troughs, shown in section in the figure (*T, T*). Into each trough dips a copper rod having two right-angle bands. These rods are connected by flexible leads, *f, f*, with the copper wires leading down through the ebonite to connect with the platinum electrodes, *E, G*. The flexible leads allow the disk *D*, which carries the copper rods, to be lifted, turned through 90° and slipped into position again when the holder is changed from one axis to the other. The troughs *T, T*, therefore, need not be disturbed during this operation. If the troughs are made long enough (about 6 in.) the holder can be tilted 40° from the horizontal in either direction.

The recording system (amplifier, Matthews oscillograph, loud-speaker and moving-paper camera) was the same as used in previous work [Löwenstein & Sand, 1930; Sand, 1937, 1938]. The rotation of the turn-table and of the tilting holder were recorded by means of a mechanical signalling device which controlled the displacement of a wire intercepting the oscillograph beam in front of the cylindrical lens of the camera.

RESULTS

The effects of angular displacement of the labyrinth about its vertical, transverse and longitudinal axes were observed in eighteen preparations. Of these, twelve were preparations of the posterior vertical ampullae, five were anterior vertical ampullae, and one was the right horizontal ampulla. Our original observation on the left horizontal ampulla of *Scyllium* was fully confirmed. The pattern of behaviour that was then described is thus typical of all the semicircular canals of the elasmobranch labyrinth. The ampullary sense organs are constantly in a state of spontaneous activity when the labyrinth is at rest, and discharge a stream of asynchronous impulses along their nerves. These discharges are increased during angular displacement in the plane and direction appropriate for each individual semicircular canal, and they are inhibited during displacement in the direction antagonistic to that which excites them.

In Fig. 3 are shown records of the responses of the right horizontal ampulla to horizontal rotation. Record A shows the spontaneous resting discharge, and the response to a clockwise (ipsilateral) rotation through an arc of 90° , signalled by the upward displacement of the white line. The time intervals at the bottom of the record are seconds. The average rate of rotation in record A was therefore of the order of one revolution

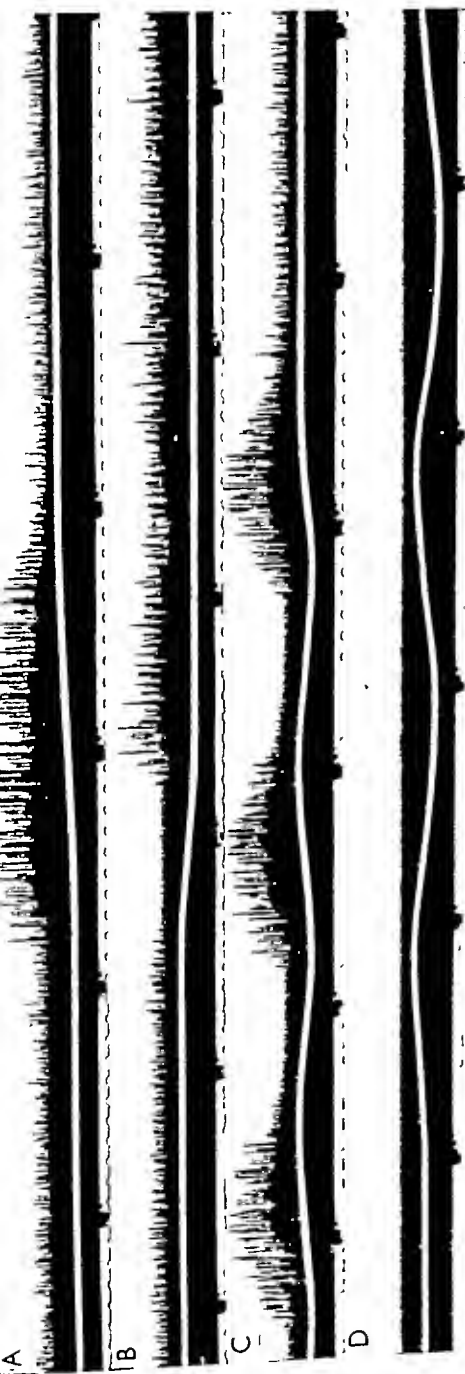


Fig. 3. Records of the responses of the right horizontal ampulla to horizontal rotation. A. Spontaneous resting discharge and response to clockwise rotation. B. Spontaneous resting discharge, response to anticlockwise rotation, and after-discharge. C. Response to consecutive clockwise and anticlockwise swinging of the turn-table through an arc of about 60° . D. Control record after crushing of nerve. Explanation of white signal-line in text. Time intervals = 1 sec. The records read from left to right.



Fig. 4. A. Response of left posterior ampulla to consecutive backwards and forwards tilting about the transverse axis. B. Control record after crushing of nerve. Explanation of white signal-line in text. Time intervals = 1 sec. The records read from left to right.

in 6 sec. Record B shows the inhibition of the discharge during 90° of rotation in the opposite sense (anticlockwise). In record C the turn-table was swung to and fro through an arc of about 60°, producing an alternation of excitation and inhibition of the discharge, and, at the end of this record, where the turn-table is brought to rest, the spontaneous discharge is re-established. Control record D was taken after the ampulla had been crushed, the nerve itself remaining intact on the electrode. The amplification remained constant for all four records.

Fig. 4 shows a similar record from the left posterior ampulla during tilting backwards and forwards about the transverse axis through an arc of about 70°. Downward displacement of the white line indicates backward tilting of the labyrinth, which is excitatory in the case of the posterior ampulla, upward displacement of the line indicates forward tilting which is inhibitory. A record from the ampulla of an anterior canal would look exactly the same with the one essential difference that in that case forward tilting would be excitatory and backwards tilting inhibitory. Technically the tilting holder was less satisfactory than the turn-table, for there was a slight roughness in its movement which produced a microphonic unsteadiness of the base line, which is still shown in the control record B, taken after the ampulla had been crushed.

After-effects. In Fig. 3, record B, it will be observed that the inhibitory response to the rotation of the labyrinth is followed by an outburst of discharge which is significantly greater than the resting discharge before the beginning of the rotation. Such an after-discharge has appeared in the majority of our experiments, but was considerably more pronounced in some preparations than in others. In those cases where the after-discharge was prominent, the converse phenomenon was also observed, namely a silent period following the response to an excitatory stimulation. This was strikingly reminiscent of the silent period which occurs in single-fibre preparations of the lateral line following stimulatory perfusion of the canal [Sand, 1937].

The dynamic responses of a semicircular canal to rotation undoubtedly depend upon the integrity of the entire structure. Any damage which interferes with the continuity and free circulation of the endolymph inside the canal abolishes the dynamic responses. Preparations in which the canal was ligated or cut, or in which the sacculus was perforated ceased to respond either with excitation or inhibition to angular displacements. The spontaneous discharge in such damaged preparations, however, continued unaffected. The interpretation of these facts depends on the recognition of the spontaneous discharge as a property of

acoustico-lateral receptors in general, a property which is independent of the peculiar structural features of the labyrinth. It continues as long as the sensory elements of the crista are themselves intact and in good physiological condition. But the complete apparatus of semicircular canal, ampulla and cupula determines and restricts the mechanical conditions which can bring about a deformation of the cupula, with the resulting changes in the impulse discharges.

In Table I are summarized the responses of all the semicircular canals to rotation about the three primary axes. It will be observed that the horizontal canals are affected only by rotation about the vertical axis,

Table I. Responses of the six semicircular canals to angular displacements about the three primary axes. ● excited; ⊗ inhibited; ○ unaffected. ant. vert.=anterior vertical; post. vert.=posterior vertical.

Semicircular canal	Longitudinal axis		Rotation about the Transverse axis		Vertical axis	
	Right	Left	Forwards	Backwards	Clockwise	Anticlockwise
Right ant. vert.	●	⊗	●	⊗	⊗	●
Left ant. vert.	⊗	●	●	⊗	●	⊗
Right post. vert.	●	⊗	⊗	●	●	⊗
Left post. vert.	⊗	●	⊗	●	⊗	●
Right horizontal	○	○	○	○	●	⊗
Left horizontal	○	○	○	○	⊗	●

whilst the four vertical canals respond either with excitation or inhibition to any rotation about any of the three primary axes. For example: the right anterior vertical canal is excited (i) by tilting towards the right about the longitudinal axis, (ii) by forward tilting about the transverse axis, and (iii) by anticlockwise turn-table rotation about the vertical axis.

It may be emphasized that the electrical responses in the three cases are entirely identical with the possible exception of differences in threshold which could, however, not be analysed.

The table shows further that the four vertical canals are functionally grouped in pairs, the grouping differing in the three types of rotation. Thus, during rotation about the longitudinal axis (tilting sideways) they may be described as *laterally synergic*, during rotation about the transverse axis (tilting forward and backward) they are *transversely synergic*, and during rotation about the vertical axis (turn-table rotation) they are *diagonally synergic*. For example: tilting towards the right excites the right anterior and posterior canals (lateral synergy), tilting forwards

excites the right and left anterior canals (transverse synergy), and clockwise turn-table rotation excites the left anterior and the right posterior canal (diagonal synergy).

DISCUSSION

An important difference is found to exist between the mode of function of the horizontal and of the vertical canals. In the horizontal canals excitation occurs when the ampulla follows the canal during angular displacement, the stimulus being ampullopetal inertia movement of endolymph. In the vertical canals excitation is caused by angular displacements in which the ampulla is leading, the stimulus being ampulofugal inertia movement of endolymph. From the days of Breuer and Ewald this difference between the two groups of canals has been repeatedly postulated by many authors [cf. Ross, 1936]. No obvious morphological character is known that could account for this functional difference.

In attempting to correlate the observed electrical activity of the individual semicircular canals with the compensatory effector responses occurring during angular displacement of an animal, the dynamic reflex responses of the eyes may serve as suitable test-reactions. A detailed description of these reflexes may be found in a recent review on the subject [Löwenstein, 1936]. Only a few facts need, therefore, be described here. Sideways tilting is accompanied by so-called vertical eye deviations which are brought about mainly by the action of the superior and inferior rectus muscles of the eye. Forward and backward tilting leads to rotatory eye deviations which are due to the action of the superior and inferior oblique eye muscles. These reactions are usually considered to be correlated with the integrated activity of the vertical semicircular canals. The eye reaction following rotation around a vertical axis is the so-called horizontal eye deviation followed by nystagmus. This characteristic reflex is brought about by the action of the internal and external rectus muscles, and has generally been considered to be evoked by the activity of the horizontal canals. It will be recalled that our experiments have shown that, apart from the horizontal canals all four vertical canals react to horizontal rotation. The question arises, therefore, whether this response of the vertical canals is in any way involved in the mechanism of the horizontal eye responses.

It would be quite plausible to assume that the pattern of afferent impulses correlated with diagonal synergy of the vertical canals might be received by the centre as a signal specific for horizontal rotation, as

opposed to the patterns of lateral and transverse synergy, denoting tilting about the longitudinal and transverse axes. If this be so, the horizontal canals might well be considered to be superfluous. However, it has been shown by elimination experiments in the pike, *Esox lucius* [Löwenstein, 1937], that section of the nerves supplying both horizontal ampullae totally abolishes the horizontal eye reactions, although the function of the vertical canals is completely unimpaired by this operation. It is clear, therefore, that the response to horizontal rotation does not enable the vertical canals themselves to evoke the horizontal eye reactions. The following considerations may suggest, however, in what way this response might be involved in the final adjustment of the mechanism of these reflexes.

Table II shows a number of diagrammatic representations of the labyrinth viewed from above with the six canals projected into one plane. A, B, C, and D indicate the state of excitation and inhibition arising on the outset of each one of the four tilting movements carried out in our experiments. Column 2 shows the eye reflex that would be observed in a reflex test, and column 3 the eye muscle whose contraction may be considered to be the main cause of that reflex. E shows a similar picture of what happens on the outset of clockwise turn-table rotation about a vertical axis. In order to get an idea of what might be the reflex effect of the diagonally synergic excitation and inhibition of the four vertical canals during this horizontal rotation, it may be permissible to abstract from A, B, C, and D a correlation between the excitation of a given canal and the reflex contraction of eye muscles to which this can give rise. This is shown in scheme F. All four vertical canals are represented there in the excited state, and against them are noted the muscles of the right eye whose contractions can, according to A-D be correlated with that state of excitation. At the same time the oblong rectangles indicate the three types of synergy observed in our experiments.

The result of this analysis is strikingly satisfactory. It shows that during sideways tilting (lateral synergy) the contraction of the superior rectus muscle (and, of course, the simultaneous relaxation of its antagonist, the inferior rectus, omitted for the sake of clarity) brings about the vertical eye deviation. At the same time the other pair of antagonists, the superior and inferior obliqui are both made to contract simultaneously. Similarly, during forward tilting (transverse synergy), the contraction of the inferior oblique (and the simultaneous relaxation of the superior oblique, not included in the scheme) bring about the rotatory deviation of the eye and at the same time the antagonistic

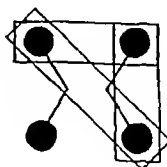
Table II. Correlation between the responses of the six semicircular canals to angular displacement, and the ensuing reflex contractions of the six eye muscles. ● excited; ⊗ inhibited; ○ unaffected; *a.v.* anterior vertical canals; *p.v.* posterior vertical canals; *l.h.* left horizontal canal; *r.h.* right horizontal canal.

Response of canals to	Reaction of right eye	Contracting muscle, mainly responsible for reaction
A. Tilting right 	Vertical deviation upwards	Superior rectus
B. Tilting left 	Vertical deviation downwards	Inferior rectus
C. Tilting forwards 	Rolling upwards	Inferior obliquus
D. Tilting backwards 	Rolling downwards	Superior obliquus
E. Clockwise rotation 	Horizontal deviation forwards	Internal rectus

F. Correlation between excitation of the vertical canals and contraction of the superior and inferior recti and obliqui

Inferior rectus
Inferior obliquus

Inferior rectus
Superior obliquus



Superior rectus
Inferior obliquus

Superior rectus
Superior obliquus

superior and inferior recti are simultaneously made to contract. This simultaneous contraction of pairs of antagonistic eye muscles not directly concerned in the reflex movement at progress, has actually been observed in the rabbit by Lorente de No [1931], who ascribes to it the function of providing a fixed pivot around which the actual reflex movement of the eye can take place. In the case of horizontal rotation about a vertical axis Lorente de No [1931, p. 111, Fig. 10] shows that during the nystagmic activity of the internal and external recti, both the superior and inferior recti and the superior and inferior obliqui contract simultaneously. Exactly the same results follow from our analysis. Scheme E shows the state of excitation and inhibition of the canals during clockwise rotation. Choosing the excited vertical canals (left anterior and right posterior) and transferring the pattern of their diagonal synergy to scheme F, we see that the theoretical result would, in fact, be the simultaneous contraction of the two antagonistic pairs of eye muscles (superior and inferior recti, superior and inferior obliqui). It may well be assumed, that, if these contractions do occur in our animal, the effect would be the creation of a pivot around which occurs the horizontal eye deviation with nystagmus, which is brought about by the antagonistic working of the horizontal eye muscles, and which is solely due to the activity of the two horizontal canals. It may be emphasized that it is not known whether one is really justified in coordinating in this simple way the activity of individual semicircular canals with that of individual effector muscles. It has been claimed [Lorente de No, 1931] that in mammals the final pattern of reflex responses is largely due to the highly complex integrating activity of the centre. We have, nevertheless, thought it useful to carry out and describe at length the above analysis, in order to demonstrate that much of the complex picture of the integrated labyrinthine reflex responses to rotation can be explained more or less exclusively on the basis of the activity of the sense organ, once the exact nature of that peripheral activity has been cleared up by means of the electrophysiological method used in our experiments.

SUMMARY

1. The individual behaviour of the semicircular canals of *Raja* has been investigated in surviving preparations of the isolated labyrinth by the oscillographic method. The effects of rotation or tilting about the vertical, longitudinal and transverse axes were determined.
2. There is a spontaneous discharge of sensory impulses from each ampulla when the labyrinth is at rest.

3. During angular displacement in the appropriate direction the discharge of impulses is increased or inhibited.

4. The horizontal canals respond to rotation about the vertical primary axis, but are unaffected by rotations about the two horizontal primary axes.

5. The anterior and posterior vertical canals respond to rotation about all three primary axes.

6. During rotation about the horizontal longitudinal axis the four vertical canals are laterally synergic and during rotation about the horizontal transverse axis they are transversely synergic. During rotation about the vertical axis the four vertical canals are diagonally synergic.

7. The occurrence of an after-discharge following inhibitory rotation and of a silent period following excitatory rotation has been observed.

8. Ligating or cutting the canal or perforation of the sacculus abolishes the dynamic responses to rotation, but leaves the spontaneous discharge unaffected.

9. The integrated action of the six semicircular canals during rotation about the three primary axes are analysed in relation to the eye-muscle reflexes evoked by these rotations.

One of us (O. L.) wishes to thank the Physiological Society for the use of their table at the Laboratory of the Marine Biological Association, Plymouth, and his thanks are also due to the Director and Staff of that laboratory for their kind hospitality and help.

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A PUMP OF SMALL INTERNAL VOLUME

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THIS pump was designed for a perfusion apparatus, and one of the main requirements was a small internal volume. By dispensing with conventional forms of valve and making use of the principle that fluid can be propelled along a flexible tube by an occlusion or constriction

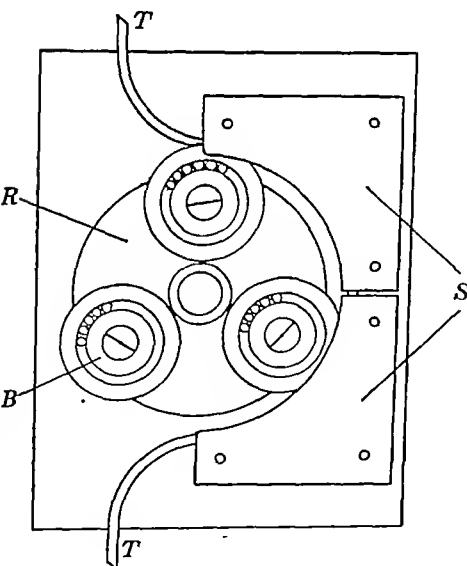


Fig. 1. Front view.

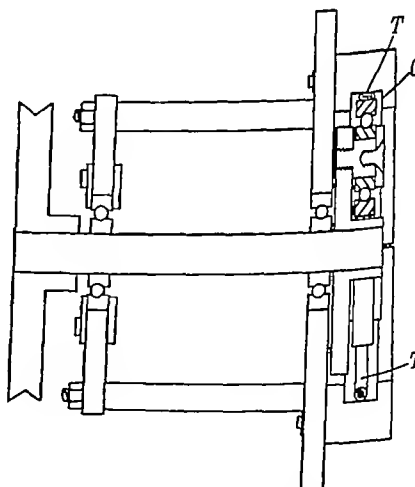


Fig. 2. Side view, partly sectional.

passing along it [Bayliss & Müller, 1928], the requirements of minimum volume are met. In this pump, small bore rubber tubing lies in a semi-circular groove, along which rollers continually pass, so pushing the contained fluid along.

The rollers consist of ballraces (*B*) 22 mm. in diameter, mounted on a rotor *R*, 48 mm. in diameter. The ebonite supporting blocks *S* have machined in them a groove in which run the outer rings of the ballraces, Fig. 2, *G*. The inner surface of the groove is about 1.5 mm. greater in radius than the distance from the centre of the rotor to the outer edge of the races. This groove accommodates the length of small bore rubber tubing *T*, shown in section in Fig. 2, which is suitably supported at its ends and contains the fluid to be pumped. The rotor spindle is mounted on ball bearings and driven by a pulley. To secure the necessary precision in locating the supporting blocks in relation to the rotor and races, the blocks are adjustable over a small range by having their fixing screws pass through fairly large holes, covered by washers, in the baseplate. The rubber tubing is easily removed when necessary, its useful life depending on the speed at which the rotor runs. The metal parts, excluding spindle and races, are brass, and can with advantage be chromium plated to resist corrosion.

Grateful acknowledgement is made to the Medical Research Council, who partly defrayed the cost of constructing the pump.

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The release of adrenaline might be the direct outcome of the lytic action of lysolecithin, in which case it would be demonstrable in *in vitro* experiments by the action of lysolecithin on a suspension of cells and cell debris from the adrenals. It might, however, be effected by the intermediate liberation of histamine, which would then in its turn act as a secretory stimulus. Since histamine is known to cause an output of adrenaline in cats and not in rabbits, lysolecithin, if acting in this way, would have no secretory action on the adrenal medulla of rabbits.

METHODS

In cats we have examined the output of adrenaline by the adrenals *in situ* and by isolated perfused adrenals. A few experiments were made in rabbits. In cats the brain and spinal cord were pithed under ether-chloroform anaesthesia. The rabbits were anaesthetized by intravenous injection, through an ear vein, of chloralose. For the *in situ* experiments the abdominal viscera were removed and the drugs injected through a cannula tied into the central stump of the coelic artery, the abdominal aorta and inferior vena cava having been tied below the adrenals. The method has been described by Feldberg, Minz & Tsudzimura [1934].

Perfusion of the cat's left adrenal. Perfusion was carried out with oxygenated Locke solution from a Dale-Schuster pump through a cannula tied into the central stump of the coelic artery. In order to keep the temperature of the inflowing fluid constant, a glass T-piece was inserted into the rubber tubing near the cannula and attached to an overflow. The perfusion pressure was regulated by the height of the overflow, and the temperature by an increase or decrease of the stroke of the pump. The perfusion pressure was kept between 60 and 90 mm. Hg, and the rate of perfusion between $1\frac{1}{2}$ and $2\frac{1}{2}$ c.c. per min. The venous outflow was collected from a cannula tied into the adrenal vein. The experiment was performed on eviscerated spinal cats. From the origin of the coelic artery the side branches of the aorta were tied and cut for a length of about $1\frac{1}{2}$ in., leaving the tissue between the adrenal and the aorta undisturbed. A corresponding piece of the inferior vena cava was similarly cleaned. The left renal vessels were tied and cut near the hilum in order to leave a small arterial branch open which often originates from the renal artery and supplies the adrenal. The tissue at the lateral side and at the back of the gland was cut between numerous double ligatures, so that at the end of the preparation the adrenal was attached to the prepared piece of aorta and vena cava only, all other connexions with the body having been severed. When the splanchnic nerve was

of adrenaline from the suprarenals and were absent after their removal. Doses of $1\mu\text{g.}$ of venom or less were ineffective. The effect of $5\text{--}10\mu\text{g.}$ was sometimes pronounced; usually, however, larger doses were required. The rise in pressure started after a latent period of $40\text{--}60\text{ sec.}$, and was sometimes preceded by a fall due to the depressor action of the venom. In the experiment of Fig. 1 the injection of $4\mu\text{g.}$ of venom (at *B*) after a latency of about 1 min. caused a small rise of pressure lasting a few minutes. The subsequent injection of $150\mu\text{g.}$ (at *C*) produced an initial strong output of adrenaline raising the blood pressure to about 150 mm. Hg. followed by a prolonged period of a more moderate and slowly decreasing output. The blood pressure had not returned to its original level 50 min. after the injection, indicating that the output

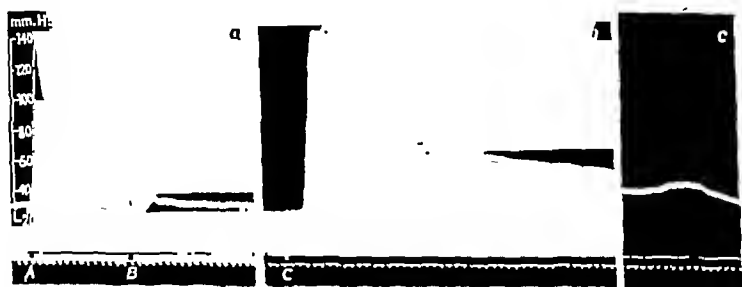


Fig. 1. Arterial blood pressure of 3.2 kg. pithed cat; eviscerated; renal vessels tied at hilum; abdominal aorta and vena cava inferior tied below the adrenals. At *A* intra-venous injection of $5\mu\text{g.}$ adrenaline; at *B* and *C* arterial injection of 4 and $150\mu\text{g.}$ bee venom respectively. Between *b* and *c* interval of 25 min. Time in half minutes.

of adrenaline had not come to an end within this period. Sometimes the return of the blood pressure to its pre-injection level did not proceed steadily, but was interrupted by irregular rises of pressure. When the injections of bee venom were repeated the effects became progressively smaller.

The output of adrenaline was associated with a loss of adrenaline from the adrenals. For instance, when the right adrenal was removed before, and the left after two or three injections of $100\text{--}200\mu\text{g.}$ of venom, the yield of adrenaline obtained on saline extraction from the left gland was $20\text{--}30\%$ less than that obtained from the right gland.

Bee venom, even in doses which caused a moderate output of adrenaline, decreased the response of the adrenals to a subsequent stimulation of the splanchnic nerves. The onset of the rise of pressure resulting from the secreted adrenaline was delayed, the rise proceeded more gradually

and was less pronounced. In some experiments splanchnic stimulation became ineffective.

Post mortem the adrenals removed after the injections of venom had a spotted appearance resulting from numerous haemorrhages. Histologically there was local and diffuse polymorphonuclear leucocytic infiltration, capillary congestion, haemorrhages, lysis of the red blood cells and some destruction of cortical cells. There were no visible changes or abnormalities in the medullary cells.

Cobra venom. The effect on the suprarenal medulla resembled that of bee venom. In Fig. 2 (at *A* and *D*) are seen the responses to two

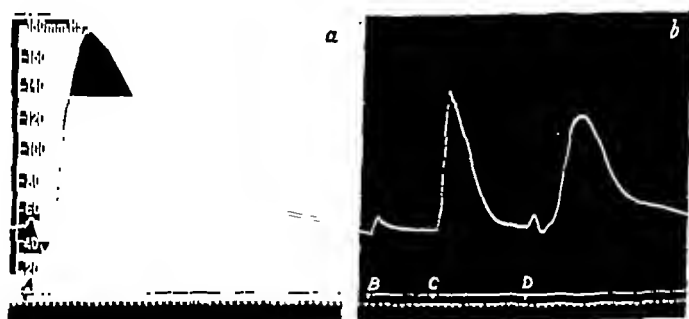


Fig. 2. Arterial blood pressure of 2.7 kg. pithed cat; eviscerated; vessels tied as in experiment of Fig. 1. At *A* and *D* arterial injection of 140 μ g. cobra venom; at *C* intravenous injection of 5 μ g. adrenaline; at *B* arterial injection of 0.5 c.c. saline solution. Time in half minutes.

arterial injections of 140 μ g. of venom (in 0.4 c.c. volume). After removal of the adrenals the arterial injections were purely depressor in action.

The *post mortem* appearance of the adrenals resembled that described for bee venom. There was also a diminution of the adrenaline content of the medulla.

Lysolecithin. Its injection into the central stump of the coeliac artery in a concentration of 1 in 1000 or stronger caused, after a latency of 30–60 sec., a rise in arterial blood pressure lasting from a few minutes to 2 hr. and being associated with acceleration of the heart beat. These effects resulted from an output of adrenaline from the adrenals and were absent when these had been removed or when the injections were made intravenously. In these cases lysolecithin produced only its depressor action. The effects of two arterial injections of 8 mg. of lysolecithin are

shown in Fig. 3, at *A* before, and at *B* after removal of the left adrenal, the right one having been removed before the beginning of the experiment.

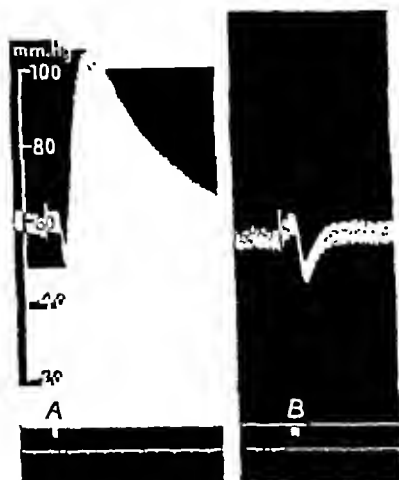


Fig. 3. Arterial blood pressure of 2.8 kg. pithed cat; eviscerated; vessels tied as in experiment of Fig. 1; right adrenal removed. At *A* and *B* arterial injection of 8 μ g. lysolecithin in 0.4 c.c. saline solution. Between *A* and *B* removal of left adrenal. Time in half minutes.



Fig. 4. Arterial blood pressure of 2.8 kg. pithed cat; eviscerated; vessels tied as in experiment of Fig. 1. At *A* intravenous injection of 5 μ g. adrenaline; at *B*, *C* and *D* arterial injection of 8 μ g. lysolecithin in 0.4 c.c. saline solution. Time in half minutes.

The effect of a first large dose of lysolecithin was usually weaker and more evanescent than that of a second similar one. The difference was sometimes pronounced. In the experiment of Fig. 4 it consisted mainly in the duration of the output of adrenaline. After the first injection (at *B*) the blood pressure had returned to about its original level within 10 min., whereas after the second injection (at *C*) it took over 30 min. Subthreshold doses of lysolecithin injected repeatedly usually remained ineffective, but rendered the medulla more sensitive to a subsequent

larger dose. In the experiment of Fig. 5 five ineffective injections of $2.5 \mu\text{g.}$ of lysolecithin, in 0.5 c.c. fluid, were given; the effect of the last one is seen at *A*. The medulla of the right adrenal—the left one having been removed before the beginning of the injections—responded now to an arterial injection of $8 \mu\text{g.}$, in 0.4 c.c. (at *B*) with a strong and long lasting output of adrenaline. The output had not come to an end about 2 hr. after the injection when the adrenal vein was tied (at *C*) and the gland removed (at *D*). In some experiments the prolonged output of adrenaline proceeded less regularly. The blood pressure tracing showed irregular rises of 30–80 mm. Hg, lasting for several minutes and following one another over a period of 2 hr. or longer.

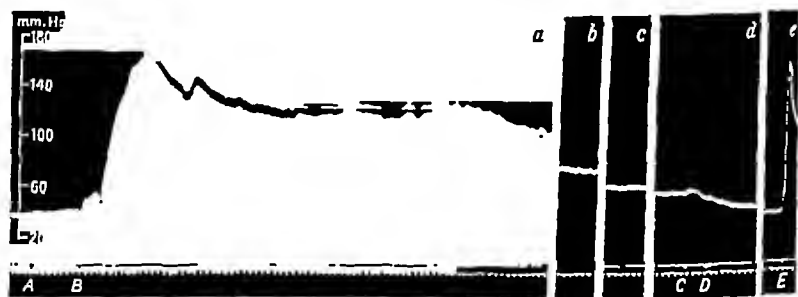


Fig. 5. Arterial blood pressure of 3.9 kg. pithed cat; eviscerated; vessels tied as in experiment of Fig. 1; right adrenal removed. At *A* and *B* arterial injection of 2.5 and $8 \mu\text{g.}$ lysolecithin respectively. At *E* intravenous injection of $5 \mu\text{g.}$ adrenaline. At *C* vein of left adrenal tied near vena cava; at *D* left adrenal removed. Between *a* and *b* and *b* and *c* interval of 30 min., between *c* and *d* of 15 and between *d* and *e* of 5 min. Time in half minutes.

When large doses of lysolecithin were injected more than twice the output of adrenaline became delayed and smaller. It could even become negligible, as shown in the experiment of Fig. 4 at *D*. The effect of splanchnic stimulation on the adrenal medulla was similarly altered. In some experiments splanchnic stimulation became ineffective. A weakening effect on the splanchnic response was already observed after a single injection of lysolecithin.

The decrease in the response to lysolecithin did not result from a depletion of adrenaline in the medulla. Although repeated large injections lowered the adrenaline content, the loss did not amount to more than 30 %. In the experiment of Fig. 5, for instance, the right adrenal which was removed at the beginning of the experiment yielded $193 \mu\text{g.}$ of adrenaline on saline extraction. The left gland removed after

the lysolecithin injections yielded 143 μ g. The corresponding figures for the adrenaline content of the adrenals in experiment Fig. 4 were 150 and 112 μ g. respectively.

Post mortem the adrenals removed after the lysolecithin injections showed the same changes as those described for bee and cobra venom.

Perfusion of the left adrenal.

The venous perfusate contained detectable amounts of adrenaline. During the first 10–15 min. after the beginning of the perfusion, the output of adrenaline per minute amounted to 0.8–1.5 μ g. per min. It then decreased quickly and fell within 40–60 min. to between 0.1 and 0.15 μ g. per min. (see Figs. 7, 8). Usually the output remained practically constant at this level for the next hour, or it showed a further gradual decline so that the adrenaline concentration in the venous perfusate eventually became too low to be detected by the blood-pressure method. The drugs were injected when a low constant output had been reached.

Some fluid always leaked from the tissues, and this was collected and assayed separately. It contained no detectable amounts of adrenaline. This leakage fluid increased somewhat as perfusion was continued. During the first half hour of perfusion less than 2 c.c. and sometimes less than 1 c.c. were collected. After 1½ hr. perfusion the leakage fluid sometimes amounted to 2 c.c. in 15 min., and further increased slowly as perfusion was continued.

Stimulation of the splanchnic nerve caused a large increase in the output of adrenaline. At the end of the stimulation the output returned quickly to its original low level. In a few experiments the amount of adrenaline secreted by a single maximal impulse was determined from the total amount secreted during a given number

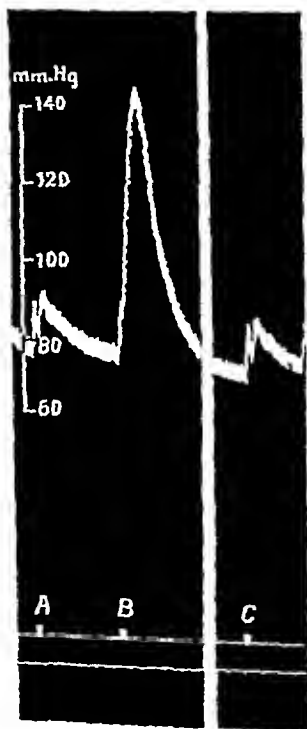


Fig. 6. Arterial blood pressure of pithed cat; injections of 0.5 c.c. perfusate from perfused cat's adrenal collected before (A) and after (B and C) 1 μ g. acetylcholine chloride. Time in half minutes. For details see text.

of maximal stimuli applied at a rate of 1-2 per sec. It amounted to 0.05-0.1 μg .

Acetylcholine injection caused an evanescent output of adrenaline (Figs. 6, 7, 8). Fig. 6 shows on the arterial blood pressure of a cat the effects of perfusate collected before (*A*), during the first (*B*) and the second (*C*) 1½ min. after an injection of 1 μg . of acetylcholine chloride into a perfused suprarenal. The output of adrenaline from this injection is plotted in tracing of Fig. 7 at *A*. Sometimes the increased output of

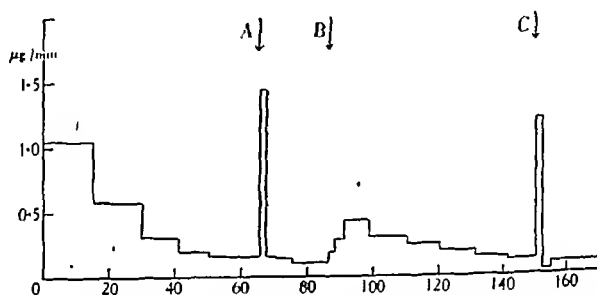


Fig. 7. Output of adrenaline from perfused left adrenal of a 2.8 kg. cat. At *A* and *C* injection of 1 μg . acetylcholine chloride; at *B* injection of 0.5 μg . lysolecithin. Ordinates: output of adrenaline in μg . per min.; abscissae: time of perfusion in minutes.

adrenaline was followed by a short period in which the output fell below the original "resting level" (see Fig. 7 at *C*). With prolonged perfusion the sensitivity of the gland to acetylcholine diminished. The values given in Table I are therefore taken from experiments during early stages of perfusion. It will be seen that the output increased with the dose injected.

TABLE I. Output of adrenaline from perfused cat's adrenal.

Amount of acetylcholine chloride injected in μg .	Output of adrenaline chloride in μg .
0.05	—
0.1	0.1; 0.5
0.25	0.1
0.5	0.3; 1.0
0.8	1.0
1.0	2.7
2.0	2.6; 5.2
5.0	4.3; 5.5
10.0	8.3

Histamine. Compared with acetylcholine the perfused adrenals are rather insensitive to histamine. A small but definite output of adrenaline could be obtained by the injection of 5 μg . of histaminedichloride.

Lysolecithin injection was followed by intense vaso-constriction which made it necessary to raise the perfusion pressure. The leakage fluid increased considerably and assumed a reddish colour due to the presence of haemolysed red corpuscles. It increased further as perfusion continued and 1 hr. after the injection it often reached 0.5–0.6 c.c. per min.

Unlike acetylcholine, *lysolecithin* caused a prolonged output of adrenaline. The difference in the response of the two drugs is illustrated in Figs. 7, 8. With doses of *lysolecithin*, such as 0.5 μ g. or less, there was only a slight increase in the output of adrenaline, which reached its maximum within a few minutes and returned to normal after 40–70 min. (Fig. 7). With larger doses of *lysolecithin* the output reached an

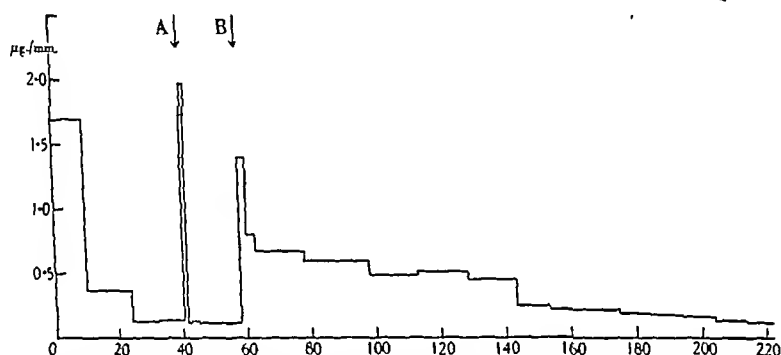


Fig. 8. Output of adrenaline from perfused left adrenal of a 3.3 kg. cat. At A injection of 1 μ g. acetylcholine chloride; at B injection of 2 μ g. *lysolecithin*. Ordinates and abscissae as in Fig. 7.

extremely high maximum within the first 2 min. and then decreased again, first quickly and later slowly. In the experiment of Fig. 8 the output of adrenaline per min. was 0.13–0.14 μ g. before, and rose to 1.4 μ g. in the first 2 min. after the injection of 2 μ g. of *lysolecithin*, but fell again in the next few minutes to less than half this value; the output then decreased slowly and did not return to its original level until 2½ hr. after the injection. The total output during this period was 61 μ g. In another experiment after the injection of 8 μ g. of *lysolecithin* 17.5 μ g. of adrenaline was secreted in the first 1½ min., the output then decreased at once and the total output in the following 8½ min. amounted only to 21 μ g. The output returned to normal after 155 min., a total of 114 μ g. of adrenaline having been secreted during this period.

The perfusate collected after the injection of *lysolecithin* contained no detectable amounts of histamine. The presence of 1 in 20 million of

histamine in the perfusate would have been detected by our assay on the isolated guinea-pig's jejunum.

That the adrenaline appearing in the perfusate after injection of lysolecithin was liberated from the adrenals was shown by the diminution of their adrenaline content. For instance, in one experiment the right adrenal which was not perfused yielded 250 μ g. of adrenaline on saline extraction. The left adrenal was perfused; during 200 min. following the injection of 8 μ g. of lysolecithin, 161 μ g. of adrenaline were collected in the venous perfusate. The adrenal then yielded only 80 μ g. of adrenaline on extraction.

Extracts of the suprarenals.

When adrenals are ground up in saline solution there remains in the debris only a fraction of its adrenaline. This is brought into solution when the debris are dissolved by the addition of lysolecithin. We proceeded as follows. The ground-up adrenals were centrifuged at 1000 r.p.m. for a few minutes to spin down the coarse material. The supernatant fluid was removed and spun down again at 3000 r.p.m. for 25 min., and a new residuum was formed. It was washed and taken up in a small amount of saline solution and divided into two equal parts. To one 1 c.c. of saline solution (the control sample), to the other 1 c.c. of a solution of lysolecithin of 1 in 50 was added. After a few minutes both parts were made up to 9 c.c. with saline solution and again centrifuged for 20 min. at 3000 r.p.m. The supernatant fluid was removed and 1 c.c. of lysolecithin 1 in 50 was added to the control and 1 c.c. of saline solution to the other sample. When both solutions were assayed on the arterial blood pressure of a cat, the sample in which the lysolecithin had been allowed to act on the cell debris produced a much stronger rise of pressure than the control solution.

Experiments on rabbits

The intravenous injection of several mg. of lysolecithin caused a steep fall in arterial blood pressure usually resulting in death. The depressor effect was associated with a rise of pressure in the pulmonary artery and was probably mainly due to pulmonary vaso-constriction. A similar injection into the central stump of the coelic artery of an eviscerated rabbit had a strong pressor effect which, when the blood-pressure level was high, was sometimes preceded by a depressor effect (Fig. 9A). With repeated arterial injections of the same dose of lysolecithin the pressor effect often varied in degree and usually became

weaker. Removal of the adrenals did not materially change the response. The effects seen in Fig. 9 were obtained after removal of the adrenals. In a few instances the pressor responses were definitely weakened and particularly shortened by the removal of the adrenals, in other experiments this procedure produced no visible change in the response. Lysolecithin, therefore, has either no or only a slight and inconstant secretory action on the adrenal medulla of rabbits, and the

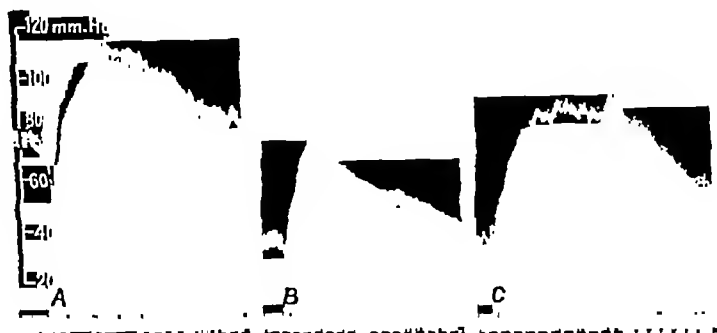


Fig. 9. Arterial blood pressure of a 3 kg. rabbit anaesthetized with chloralose and eviscerated; both adrenals removed; both vagi cut; arterial respiration. Injections into the central stump of the coeliac artery (at *A*) of 8 μ g., (at *C*) of 15 μ g. lysolecithin and (at *B*) of 0.4 μ g. histamine dichloride. Time in half minutes.

pressor effect on arterial injection must be attributed mainly to peripheral vaso-constriction. This conclusion was further substantiated by experiments in which injections were made into the iliac artery of one side, the resulting pressor response not being influenced by previous cutting of the femoral and sciatic nerves of the side of injection.

DISCUSSION

We have attributed the long lasting output of adrenaline from the cat's adrenals following the arterial injection of bee venom or cobra venom to formation of lysolecithin in the gland, because these venoms are strong phosphatases and are known to form lysolecithin in the tissues [Feldberg & Kellaway, 1938], and because we could show that lysolecithin causes a long lasting output of adrenaline from the medullary cells. In addition to the formation of lysolecithin, the formation of lysocephalin may contribute to the effects of these venoms on the adrenals.

into the tissue spaces. Evidence for the release of adrenaline by lysolecithin under physiological conditions, therefore, is more readily obtained than the release of histamine.

The circulatory effects of lysolecithin have hitherto only been described for cats and dogs. Our experiments on rabbits suggest that the depressor effect in this animal is mainly the result of vaso-constriction in the lungs. Lysolecithin, however, has some vaso-dilator action in the systemic circulation as shown by the depressor effect preceding the strong pressor response on its injection into the abdominal aorta. The fact that lysolecithin has a weak vaso-dilator and a strong vaso-constrictor action in the systemic circulation of the rabbit agrees with what is known about the vascular effects of cobra venom in this species.

SUMMARY

1. *In cats* bee venom and cobra venom cause a long lasting output of adrenaline from the adrenals if injected into the central stump of the coelic artery after evisceration. The effect has been attributed to formation of lysolecithin in the adrenals since the venoms are strong phosphatases forming lysolecithin in the tissues, and since lysolecithin was found to cause an output of adrenaline similar to that produced by the venoms. The output of adrenaline was associated with a diminution of adrenaline in the adrenals. After repeated large doses of venom or of lysolecithin, the medullary cells became irresponsive to these or to other secretory stimuli.

2. The effect of lysolecithin has been studied on the isolated cat's adrenal perfused with Locke solution. Lysolecithin caused an output of adrenaline which lasted sometimes for more than 2 hr. and amounted to more than 0.1 mg. The long lasting output is regarded as a response of the medullary cells to injury, and has been contrasted with the strong but evanescent output produced by acetylcholine or by nerve stimulation.

3. Lysolecithin causes a release of adrenaline in *in vitro* experiments from a suspension of ground-up cellular material of the cat's adrenal.

4. *In rabbits* lysolecithin has either no or only a slight and inconstant secretory action on the adrenal medulla. Its intravenous injection causes a fall of arterial blood pressure associated with a rise of pressure in the pulmonary artery. Its injection into the abdominal aorta causes, even after removal of both adrenals, a rise in arterial blood pressure (peripheral vaso-constriction), sometimes preceded by a depressor effect (peripheral vaso-dilatation).

I should like to make grateful acknowledgement to Dr J. Hart-Mercer (Cambridge) for examining the histological preparations of the suprarenals, to Dr C. H. Kellaway (Melbourne) for supplying me with cobra venom and to Dr M. Guggenheim, Dr Winterstem and the firm Hoffmann-La Roche (Basle) for their great help in supplying me with bee venom, lysolecithin, acetylcholine chloride and histamine dichloride, and to Dr Guggenheim further for his stimulating interest in the experiments described in this paper.

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IONS AND ADRENERGIC TRANSMISSION IN THE RABBIT'S EAR¹

By CHANG-SHAW JANG

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(Received 17 June 1940)

POTASSIUM [Brown & Feldberg, 1936] and calcium ions [Bronk, Larrabee, Gaylor & Brink, 1938; Harvey & MacIntosh, 1940] have been found to exert an important influence on synaptic transmission in the cat's superior cervical ganglion. Evidence is not lacking that, on the cholinergic and adrenergic transmission at the postganglionic terminals, these ions may exert an equally important influence. But so confusing is the literature, which will be dealt with briefly later, that a reinvestigation of this matter with better methods seemed desirable. As the title implies, this report deals only with the effect of cations on adrenergic transmission. Gaddum & Kwiatkowski's rabbit-ear preparation was chosen for the present study for the following reasons: (1) In this preparation, electrical stimulation can be applied directly to the postganglionic sympathetic fibres, which appear to consist of pure adrenergic nerves, thus excluding the complication of synaptic transmission in the ganglion which was involved in some of the earlier experiments, in which preganglionic nerves were used for stimulation. (2) The substance liberated from adrenergic fibres of this preparation is in all probability adrenaline itself [Gaddum & Kwiatkowski, 1939; Gaddum, Jang & Kwiatkowski, 1939]; thus, by matching the effect of injected adrenaline with that of adrenergic stimulation, it is possible to study the effect of ions on the two components of the transmission in this preparation: (a) the liberation of the transmitter, and (b) the response of effector cells to the action of adrenaline or the transmitter itself.

¹ Being a part of a thesis accepted by the University of London for the degree of Ph.D.

METHODS

The rabbit's ear was perfused according to the technique previously described [Gaddum & Kwiatkowski, 1938] with slight modifications [Jang, 1939]. The normal and variously modified Locke's solutions were prepared from the same lot of distilled water which had been previously oxygenated, thus excluding the effect of unequal oxygenation in different solutions. As a general procedure the ear was first perfused with normal Locke's solution, then with various modified solutions one after another, each one being perfused until the effect of injected adrenaline and that of nervous stimulation became constant.

RESULTS

Effects of ions on adrenergic transmission. Total replacement of NaCl in the normal Locke's solution by an appropriate amount of glucose to maintain its isotonicity increased the outflow, diminished but did not abolish the effect of adrenaline and that of nervous stimulation even after perfusion for a few hours; this diminution of effects was also observed when the rate of outflow was brought down to the previous level by lowering the perfusion pressure. The Locke's solution with only half of its normal NaCl content replaced by glucose, though still causing vaso-dilatation, had little detrimental effect on the transmission.

The responses of the perfused rabbit's ear to adrenaline and to nervous stimulation were not affected in the same way by changes of potassium and calcium content of the perfusing fluid. While calcium-free Locke's solution greatly increased the vascular response to adrenaline, it decreased that to adrenergic stimulation (Fig. 1, II). Since this increased vascular sensitivity to adrenaline still persisted when both calcium and potassium were removed from the perfusing fluid (Fig. 1, III), and since a change from the normal Locke's solution to that free from both calcium and potassium also caused an increase in adrenaline action, it seems certain that it is calcium which plays the major if not the only role in modifying the vascular sensitivity toward adrenaline. Indeed, when the calcium content in the medium was tripled or further multiplied, the vascular effect of adrenaline always became smaller and smaller. The presence of potassium itself was apparently not essential; but in the presence of calcium, potassium appeared to counteract to some extent the unfavourable effect of the former. Thus an increase of potassium in the fluid sometimes slightly increased (Fig. 2) and its removal almost always lessened the adrenaline action in the perfused vessels.

On the other hand, the effect of adrenergic stimulation was apparently more affected by changes in potassium than in calcium content of the perfusing fluid. When both potassium and calcium were absent (Fig. 1, III)

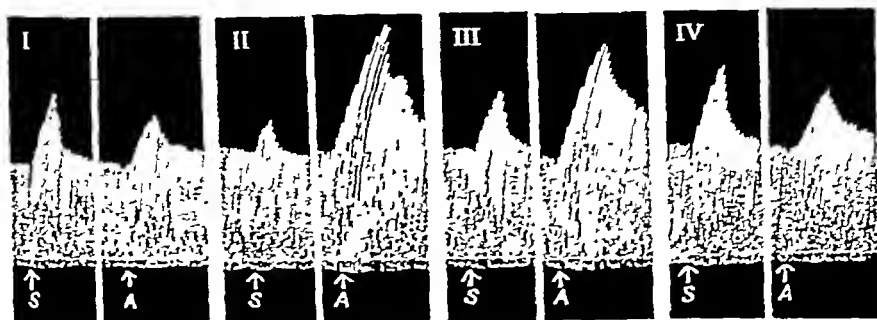


Fig. 1. Effects of successive perfusion with normal Locke (I), Ca-free Locke (II), Ca- and K-free Locke (III) and K-free Locke (IV) on the outflow from a rabbit's ear. The height of the record in this and the following figures is proportional to the time interval between successive drops. *S*, stimulation of adrenergic nerves, 3 sec., 36 shocks per sec.; *A*, injection of 0.1 c.c. of adrenaline (3×10^{-7}).

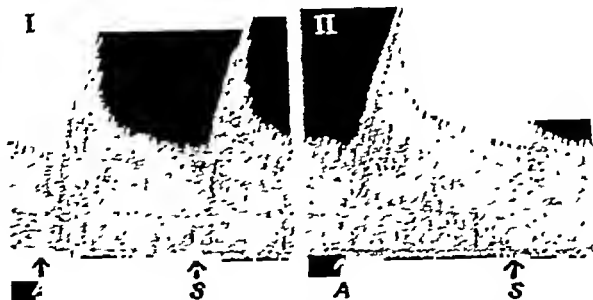


Fig. 2. Outflow from a rabbit's ear, perfused with normal Locke (I) and then with Locke containing KCl in three times the normal amount (II). *A*, adrenaline (10^{-7}); *S*, stimulation, 2 sec.

the effect of adrenergic stimulation was slightly affected; but an introduction of potassium (II) at once much diminished it. The introduction of calcium (IV) definitely increased the effect of adrenergic stimulation, but not so markedly as it depressed the adrenaline action. In the normal Locke's solution, any increase of potassium content up to three times normal invariably much diminished the effect of adrenergic stimulation (Fig. 2), but a similar increase of calcium did not produce a regular effect. In general, the nervous effect often slightly increased if the

amount of calcium was not more than double its usual amount in the solution, but usually decreased if the calcium concentration was still higher.

When the pH (about 7.2) of the normal Locke's solution was brought up to about 8.0 by adding 0.1 % $NaHCO_3$ instead of 0.015 %, both the effect of adrenaline and that of adrenergic stimulation were equally increased (Fig. 3), indicating that higher pH of the fluid only improves the effector response to the transmitter, and that the liberation is perhaps not affected. The same improvement was obtained when 0.1–0.2 c.c. of 1 % $NaHCO_3$ were injected into the perfusion cannula.

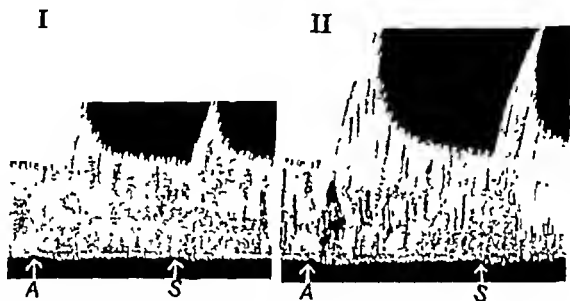


Fig. 3. Outflow from a rabbit's ear, perfused with normal Locke (I) and then with Locke containing 0.1% $NaHCO_3$ instead of 0.015 % in the normal. A, adrenaline (5×10^{-6}); S, stimulation, 1 sec.

Vascular effects of ions. All the modifications of calcium or potassium content in the perfusion medium, as described above, usually had no detectable effects on the rate of outflow. The observations recorded above were, therefore, made without changing the perfusion pressure.

On preparations perfused with isotonic glucose solution, injections of 0.2–0.5 c.c. of isotonic $NaCl$, KCl and $CaCl_2$ all had vaso-constrictor effects, KCl having the greatest and $CaCl_2$ the least. But whereas the vaso-constrictor effects of $NaCl$ and KCl were quick and transient, that of $CaCl_2$ was very slow, assuming a tonic character. During this progressive tonic constriction due to $CaCl_2$, an injection of $NaCl$ almost immediately removed the vaso-constriction, thus $NaCl$ caused a vasodilatation instead of the usual vaso-constriction. Curiously, this antagonistic property of $NaCl$ toward $CaCl_2$ was not shared by KCl , its well-known antagonist, for KCl produced its usual vaso-constriction when given during the action of $CaCl_2$.

The generally recognized vaso-dilator effect of CaCl_2 was readily observed on preparations perfused with normal saline or normal Locke, provided the solution of CaCl_2 was isotonic. The ordinary 1 % CaCl_2 solution, which is frankly hypotonic, sometimes produced vaso-constriction.

Effects of anisotonicity. Since any modification of the perfusing fluid by decreasing or increasing the total amount of its ingredients inevitably involves some change in its osmotic pressure, several experiments were made to investigate the effect of changes in tonicity of the perfusing fluid on the vessels and on the adrenergic transmission.

The changes in the tonicity were effected by withdrawal or addition of glucose instead of NaCl , because while the former is itself devoid of pharmacological actions on the perfused vessels, the latter is a vaso-constrictor agent. In the experiments with hypotonic solutions, the Locke's solution was so modified that the amount of NaCl was reduced to half the original, and the resultant hypotonicity was corrected by adding glucose (25.5 g. per litre). This solution itself caused slight vaso-dilatation, but very little change in the effects of adrenaline and adrenergic stimulation, thus differing from Witanowski's results on the frog's heart [1926].

While 10 % changes in tonicity in either direction usually had little effect on the rate of outflow and on the adrenergic transmission, profound effects were always observed, however, with changes above 10 %. Thus 20, 30 and 50 % hypotonicity in the medium caused vaso-constriction, an increase of adrenaline action and a decrease of nervous effect; on the other hand, 20, 30 and 50 % hypertonicity caused vaso-dilatation, and a diminution or even complete suppression of both effects. All these effects varied directly as the amount of deviation in either direction from isotonicity. The results are essentially in agreement with those of Dale [1913] on the isolated guinea-pig's uterus.

Although modifications of the Locke's solution in the above experiments on ions never caused anisotonicity more than 9 % in either direction, a few parallel experiments with the modified solutions, the anisotonicity of which were corrected, were repeated and the previous findings were confirmed.

DISCUSSION

The results have clearly shown that even a normal concentration of calcium ions in the perfusing fluid is unfavourable to the vascular action of adrenaline, and that changes in concentration of potassium ions are only of secondary importance. This calcium effect on the

ular action of adrenaline has been confirmed *in vivo* by Earley [40], who found that CaCl_2 diminished the pressor response of spinal cord to adrenaline and that decalcifying agents such as sodium citrate produced the opposite effect.

Results obtained by earlier workers on this aspect of calcium action present a most confusing picture. Some found that Ringer's solution deficient in calcium or containing decalcifying agents diminished the adrenaline response on perfused amphibian and mammalian vessels, and that calcium increased it [Alday-Redonnet, 1920; Medici, 1924; Solotoff, 1935]. Others found, mostly on the frog's leg preparations, that the vaso-constrictor action of adrenaline was reversed into vaso-dilator when the perfusing fluid was devoid of calcium ions [Pearce, 1913; Del Campo, 1914; Wehland, 1924; Bergengren, 1925]. But most of the latter workers obtained results essentially similar to mine [Regniers, 1926, for the earlier literature; Hayashi, 1927; Masago, 1928; Kanda, 1930; Kwiatkowski, 1931].

This unfavourable effect of calcium does not seem to be specific to the vascular action of adrenaline, since that of posterior pituitary extract is similarly affected, though less in extent [Kanda, 1930]. One might be strongly tempted to explain this calcium action by its well-known effect on the permeability of cell membranes, but calcium can also be synergistic with adrenaline on other smooth muscles [Thienes, 1926]. Since CaCl_2 , which diminishes the vaso-constrictor effect of adrenaline, is itself a vaso-dilator agent, and KCl and NaHCO_3 , which are synergistic with adrenaline, are themselves vaso-constrictor agents, the possibility exists that all these effects might be due simply to changes in the basal tone. It is interesting to note that many sympathomimetic drugs, several local anaesthetics and "sympatholytics" in concentrations slightly higher than their "sensitizing" concentrations, are themselves all vaso-constrictor agents [Jang, 1940].

The results on the favourable action of calcium ions on adrenergic stimulation are quite in agreement with the general belief among pharmacologists. Earley [1940] has recently observed that the pressor response to stimulation of the thoracic sympathetic chain is augmented by the presence of calcium. Practically all the earlier results were, in fact, explained indirectly by using decalcifying agents [Bouckaert, 1928, for earlier literature]. Schmidt [1921] stimulated the lumbar sympathetic chain of frogs, perhaps a mixture of vaso-constrictors and vaso-dilators, and Regniers [1926] stimulated the cervical sympathetic trunk in cats with the involvement of synaptic transmission; neither of them

found any improvement following the increase of calcium content of the perfusing fluid, the effect of sympathetic stimulation was only diminished if affected at all. Since synaptic transmission can be inhibited by calcium excess in the perfusing fluid [Brown & Feldberg, 1936], Regniers' results must be considered with reserve.

As calcium in the medium is definitely unfavourable to the effector response to adrenaline, the likely explanation for this favourable action of calcium on adrenergic effect would be that calcium ions would in one way or another increase the amount of adrenaline liberated. Indeed Lissák [1938] and Kwiatkowski [1938] have independently shown that excess of calcium itself liberates "sympathin" from the frog's heart and from the rabbit's ear respectively. Although this "sympathin-liberating" action of calcium can hardly be reconciled with its vaso-dilator action, it serves well to support the explanation just offered. If this explanation is correct, then the actual increase in adrenaline liberation under the influence of calcium would be necessarily much greater than it appears, because the apparent result would then only represent the algebraic sum of increased liberation and diminished sensitivity to adrenaline. These opposed effects of calcium would also explain the irregularity and unimpressiveness of its favourable action on adrenergic excitation.

In contrast to its insignificant effect on the adrenaline action, potassium possesses an inhibitory effect on the adrenergic excitation as striking as that of calcium on the adrenaline action. This effect of potassium would seem to be the result of the diminished liberation of adrenaline. In view of the findings that potassium liberates acetylcholine from both pre-ganglionic [Brown & Feldberg, 1936] and postganglionic cholinergic terminals [Beznák, 1934], the possibility that the same ion may inhibit the liberation at adrenergic nerve endings is of very great interest.

SUMMARY

1. The effects of ionic changes on adrenergic transmission have been studied in the perfused rabbit's ear preparation. Incidentally the vascular effects of ions and the effects of changes in the tonicity of the perfusing fluid were also investigated.

2. A small increase of calcium concentration decreases the action of adrenaline, but may simultaneously increase the response to nervous stimulation, presumably by increasing the amount of adrenaline liberated by the nerves. A decrease of calcium concentration has the opposite effects.

3. The effects of potassium are in the opposite direction to those of calcium, but its action on the response to adrenaline is feeble compared with its action on the response to nervous stimulation.

4. Alkalinity increases both effects.

The author wishes to thank Prof. J. H. Gaddum for his help and advice throughout the course of this work.

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EFFECTS OF VASO-DILATOR AND VASO-CONSTRICTOR SUBSTANCES ON NORMAL AND DENERVATED SPLEENS

By J. G. STEPHENS

From the Sir William Dunn School of Pathology, Oxford

(Received 17 June 1940)

THE effect of adrenaline in causing contraction of the spleen of anaesthetized dogs was shown plethysmographically by Oliver & Schäfer [1895], and confirmed on unanaesthetized dogs by Barcroft [1930]. Vaso-dilator substances such as sodium nitrite were shown by Viale [1928], Radosavljević & Sekulić [1930], and Flaum & Schlesinger [1933] to cause splenic contraction.

METHODS

For the experiments without anaesthesia, exteriorized spleens of dogs were employed, as described by Barcroft & Stephens [1927]. When immediate observations were to be made under anaesthesia, the spleen was temporarily exteriorized. Two pieces of collodion-painted cardboard, ruled in sq. mm., were placed behind the spleen, and the contiguous margins of these cardboard backgrounds were cut so as to avoid pressure on the mesentery and splenic vessels.

Pl. II illustrates this technique, the heavy ruling being in sq. cm. Changes in the size of the spleen are thus given as changes in the contour area. If necessary, such readings may be converted into approximate volume changes by employing the relative values of the quantity (spleen area)[†] [Barcroft & Stephens, 1927]. Recording of the spleen area is sufficient to demonstrate the changes which in cats and dogs are obvious to the eye.

Some degree of manipulative trauma is unavoidable, but is certainly no greater than with plethysmographic methods. Although even slight trauma upsets the sinus cycles of the spleen [Knisely, 1936*a*, *b*], it does not appear that the contractile responses are disturbed to the same extent.

3. The effects of potassium are in the opposite direction to those of calcium, but its action on the response to adrenaline is feeble compared with its action on the response to nervous stimulation.

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PL II illustrates this technique, the heavy ruling being in sq. cm. Changes in the size of the spleen are thus given as changes in the contour area. If necessary, such readings may be converted into approximate volume changes by employing the relative values of the quantity (spleen area)² [Barcroft & Stephens, 1927]. Recording of the spleen area is sufficient to demonstrate the changes which in cats and dogs are obvious to the eye.

Some degree of manipulative trauma is unavoidable, but is certainly no greater than with plethysmographic methods. Although even slight trauma upsets the sinus cycles of the spleen [Knisely, 1936a, b], it does not appear that the contractile responses are disturbed to the same extent.

This temporary exteriorization technique is probably similar to that referred to by Flaum & Schlesinger [1933] and others as a "vorgelagerte Milz", but no details of this are given in the paper cited nor has it been possible to trace them elsewhere.

Denervation of spleens. It is impossible completely to denervate spleens without also severing the blood vessels. Even when the entire splenic mesentery and all nerves and vessels, except the main splenic artery, are cut, and the artery then stripped as far as practicable, faradic stimulation of the artery produces an immediate contraction of the spleen. This is only to be expected in view of the work of Leriche & Stricker [1933]. As my experiments show, however, it is possible to produce a functional effect on the spleen by thorough nerve section, and this must be the justification for calling the procedure "denervation". The interpretation of unaltered responses by the spleen when the nerves are cut must, however, be restricted. The use of Lim's tubes [1927] would enable the blood vessels to be severed and united again.

RESULTS

Vaso-dilator substances

Both in unanaesthetized and in anaesthetized animals the contraction of the spleen produced by vaso-dilator substances was abolished by section of the nerves to the spleen.

Intravenous injection of 0.15 mg. per kg. of glyceryl trinitrate solution into unanaesthetized dogs, trained not to react to the act of injection, produced extreme contraction of the spleen within 10-25 sec.

A similar result followed immediately when the animal inhaled some of the vapour from a capsule of 0.2 c.c. of amyl nitrite broken on a cloth. In this case psychic causes also operated to produce splenic contraction. The result with amyl nitrite was photographed, however, and is shown in Pl. I. Injections of glyceryl trinitrate produced a similar response, but photographs were not made.

In order to demonstrate the effects of nerve section, use was made of the device suggested by Anrep, and previously described by Barcroft & Stephens [1927]. The nerve supply to the dorsal half only of the spleen was severed, the ventral portion remaining as a control. Text-fig. 1a shows the effect of inhalation of vapour from a 0.2 c.c. capsule of amyl nitrite on the contour of such an exteriorized spleen (dog). The denervated region did not contract. The same result was obtained in two other dogs in which the nerve supply of the ventral region of the spleen was severed. This is shown in Text-fig. 1b.



Fig. 1b.

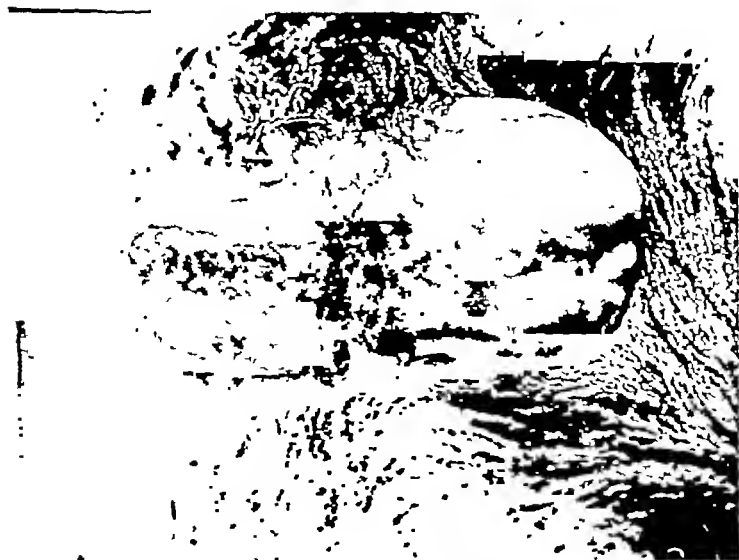


Fig. 1a.

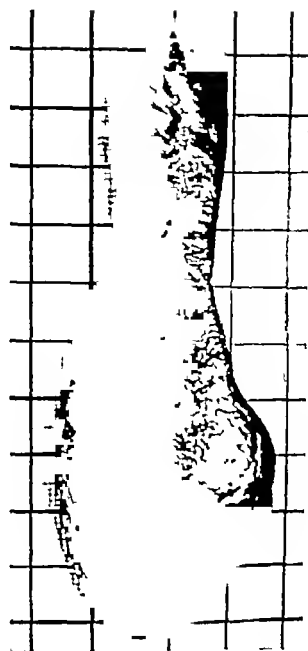
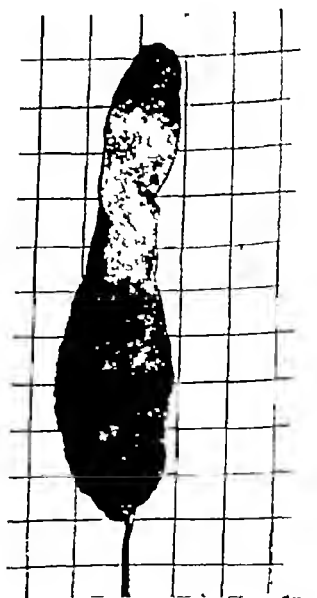
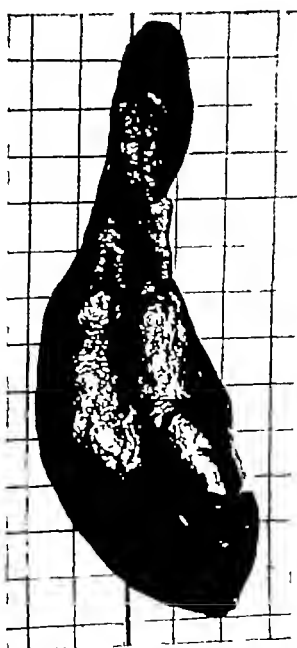


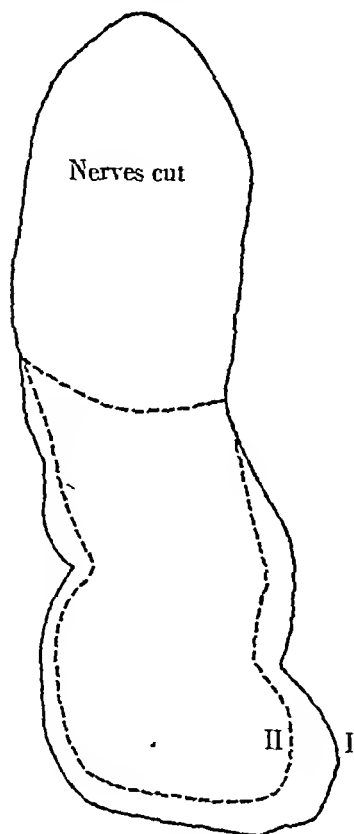
Fig 1a.



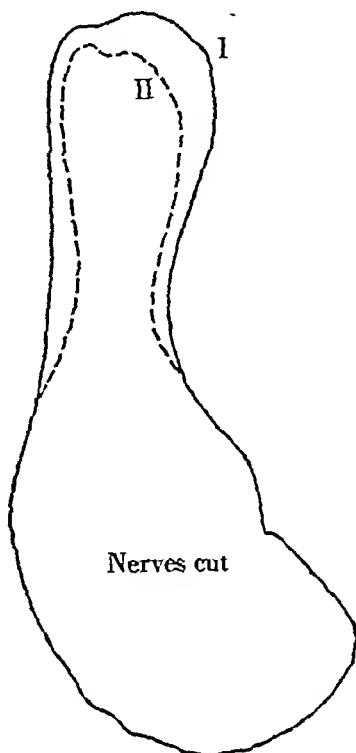
Fig. 1b.



In eleven cats and two dogs under nembutal or ether anaesthesia, inhalation of amyl nitrite gave immediately a profound contraction of the spleen which, as before, was abolished by nerve section. Pl. II, figs. 1a, b, show the effect of crushing in a cloth a 0.2 c.c. capsule of amyl



Text-fig. 1a.



Text-fig. 1b.

Text-fig. 1a, b. Contour of exteriorized spleen of dog, $\frac{2}{3}$ actual size. (a) Upper region denervated and (b) lower region denervated. I, before inhalation of amyl nitrite. II, after inhalation. The denervated region did not contract.

nitrite in the neighbourhood of the nostrils of a cat under nembutal anaesthesia. In this experiment all nerves to the ventral portion of the spleen were divided and, as shown, this abolished the splenic contraction. The denervated ventral portion of this same spleen was, however, capable of contraction, as was shown by the immediate shrinkage following adrenaline injection or faradic stimulation of the vessels. In some

experiments, the dorsal instead of the ventral region of the spleen was denervated, the effects being unaltered. The results with 0.01–0.02 g. per kg. of sodium nitrite, intravenously injected, were similar to those with amyl nitrite and glyceryl trinitrate.

Histamine (injected intravenously in doses of 0.25–1 mg. per kg. of body weight) produced, in cats under nembutal anaesthesia, contraction in both denervated and innervated portions of the same spleen, the contraction being extremely marked with the higher dosage.

Vaso-constrictor substances

Adrenaline. Adrenaline injected intravenously produces an immediate and profound contraction of both normal and denervated spleens. Pl. II, fig. 2 *a, b*, shows the effect of injecting 0.25 mg. per kg. of adrenaline hydrochloride solution into the femoral vein of a cat under nembutal, the nerves to the ventral end of the spleen being cut as before. Commercial solutions of adrenaline (containing preservative) were used and were administered within half an hour of the denervation. The contraction occurred more rapidly and was more marked in the denervated end where the surface became intensely crenated and contrasted even with the granular surface of the contracted normal spleen. This can be seen in Pl. II, fig. 2. The same result was observed in eight other such preparations. Recovery from the contraction following adrenalinic in the above dosage occurred in 10–15 min.

Tyramine. Tyramine, like adrenaline, produced contraction of both normal and denervated spleens when doses of 0.5 mg. per kg. were intravenously injected into the femoral vein of ten cats under nembutal anaesthesia. The denervated portion of regionally denervated spleens did not appear to contract more rapidly or to a greater extent than the portion with nerves intact. Recovery from the contraction after 0.5 mg. of tyramine per kg. occurred within 12–15 min., that is, in approximately the same time as for the recovery from 0.25 mg. of adrenaline per kg. The concentration of the solutions of both substances injected was the same, 1 mg. per c.c.

Quinine dihydrochloride.

Some difficulty in obtaining uniformity of response was encountered, until it was realized that the effects on the spleen of intravenous injection of quinine depended upon the rate of injection. Thus, rapid injection of 0.02 g. per kg. of body weight produced a small contraction of the spleen of nine cats and one dog. This dose is somewhat higher than the *maximum* human dose for intravenous injection which is from 0.25 to 1 g. total.

Higher dosage up to 0.05 g. per kg., rapidly administered, produced a more marked contraction of the spleens of six cats, the effect, however, being much less than that produced by adrenaline or amyl nitrite. The solution of quinine used contained 0.02 g. of quinine dihydrochloride per c.c., and its acidity was neutralized with NaOH as far as its solubility permitted, the final pH being 6.3. Control experiments were performed on four other cats in which 3 c.c. of isotonic glucose, acidified with HCl to pH 6.3, were injected, and produced no change in splenic volume.

When the quinine salt in the same concentration as above was administered very slowly over a period of 5-10 min., however, it was possible to administer a dose of 0.02 g. per kg. to five cats without apparent effect upon the spleen size, although four other cats in this series showed a contraction similar to that in response to rapid injection.

All the animals, into which the quinine was injected rapidly, showed toxic effects. The respiration slowed or temporarily stopped, and the heart became irregular. It is therefore possible that the splenic contraction was not a direct effect of the quinine, but a response to the fall in blood pressure which rapid quinine injections are known to produce [Brahmachari, 1922; Sollmann, 1936]. Accordingly, further experiments were performed in which the spleens of four cats were regionally denervated in the manner described above. Rapid injection of 0.02 g. per kg. of quinine dihydrochloride caused no contraction of the denervated region of spleen. It would thus appear that the splenic contraction in response to quinine is partly, at least, caused by the known blood pressure fall. This is discussed later. The possibility that the spleen has been deranged by the denervation procedure and other factors do not enable a more definite conclusion to be drawn from the present data.

DISCUSSION

The failure of the denervated spleen to contract under the influence of amyl nitrite and glyceryl trinitrate may mean that the splenic contraction caused by these vaso-dilators is a passive response to the fall in blood pressure. Section of the splenic nerves diminishes the tone of the splenic musculature so that it is no longer able to follow the blood pressure fall. Masuda [1927] showed that the splenic volume decreased passively when the blood pressure was lowered.

The fact that thorough section of splenic nerves, including the plexuses investing the arteries, does depress the tone of the organ is shown by the altered appearance of the spleen surface after such procedures. The surface colour of the denervated region of the spleen is darker, and its

texture smoother. The appearance contrasts with the redder, somewhat granular and less swollen appearance of the normal spleen. Nevertheless some nerve fibres survived this procedure, because the spleen contracted vigorously when the artery was stimulated faradically.

Other factors are probably involved also. The darker colour of the denervated spleen shows that the sinus cycles have been disturbed, either by the nerve section or the concomitant manipulation. This dark colour persisted for some 14 days or more in the animals with exteriorized spleens, so that trauma would not seem to have been the sole factor.

Viale & Soncini [1928] report that denervated spleens contract in response to amyl nitrite. The present experiments are opposed to this result which probably arose from incomplete section of the accessible nerves. This explanation of Viale & Soncini's observation was confirmed by severing only a few of the nerve fibres investing the splenic artery in cats and dogs. In such cases amyl nitrite produced a slow contraction of the corresponding region of the spleen, less marked than in the region with nerves intact.

The action of adrenaline in causing greater and more rapid contraction of the denervated than of the normal regions of the same spleen resembles the familiar augmented action of adrenaline on denervated plain muscle in other organs, for example, in the pupil [Meltzer & Auer 1904; Starling, 1933].

The interesting feature of the reaction of denervated spleen to adrenaline is that the increased response occurs immediately after the nerves are cut and before they have degenerated. Similarly, Hampel [1935] found that the exaggerated response to adrenaline of the cat's nictitating membrane commenced almost immediately after excision of the superior cervical ganglion.

Roth [1912] states that 0.1 g. of quinine hydrochloride injected into the jugular vein of dogs causes the spleen to contract. The weight of the animals and the rate of injection are not stated. Cushny [1936] also states that quinine produces contraction of the spleen in man and other animals, and suggests that it may arise from direct action on the musculature.

According to Brahmachari [1922] large doses of quinine, up to 1 g., in man lower the blood pressure and depress the heart. Brahmachari also showed experimentally that intravenous injections of quinine in man may cause a sharp and dangerous fall of blood pressure, especially when the injection is made rapidly. Biberfeld [1916] observed similar effects in rabbits. Sollmann [1936] states that intravenous injection of quinine

may lower blood pressure, and diminish the tone and excitability of the heart.

Because of these effects, and because of the variable action on the spleen of quinine when slowly administered and of the absence of response in denervated spleens, it is probable that the splenic contractions are largely an indirect response to the fall of blood pressure.

The contraction produced by histamine was identical in both normal and denervated regions of the spleen, and may be attributed, therefore, to a direct action on the splenic musculature.

SUMMARY

1. Vaso-dilator substances, such as amyl nitrite and glyceryl trinitrate, cause contraction of the spleens of unanaesthetized dogs and of anaesthetized cats and dogs. The effect may be passively related to the fall of blood pressure, *because* these vaso-dilators do not produce contraction of the denervated spleen.

2. Histamine produces contraction of both normal and denervated spleens.

3. Immediately after denervation of the spleen, adrenaline produces more contraction than normally.

4. Tyramine produces equal contraction of the normal and of the denervated spleen.

5. Rapid intravenous injection of quinine dihydrochloride, in doses slightly larger than the maximum human dose, contract the spleen, but this effect may be indirect and due largely to a fall of blood pressure. The response with very slow injection was variable, and contraction did not always occur.

In conclusion I wish to thank Prof. H. W. Florey for his encouragement in this work, and the Medical Research Council and the Nuffield Trustees for personal grants.

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EXPLANATION OF PLATES I AND II

PLATE I

Fig. 1. Exteriorized spleen of dog: (a) before, (b) immediately after inhalation of vapor from 0.2 c.c. capsule of amyl nitrite on a cloth. Scale: $\frac{2}{3}$ actual size. The spleen has been exteriorized for 8 months.

PLATE II

Fig. 1a. Spleen of cat. Nerves to lower half cut. b. Same spleen immediately after inhalation of amyl nitrite. The denervated region has not contracted. (Nembutal anaesthesia. Background ruled in sq. cm.)

Fig. 2a. Spleen of cat. Nerves to lower half cut. b. Same spleen immediately after intravenous injection of adrenaline hydrochloride. The denervated region has contracted more strongly than the region with nerves intact. (Nembutal anaesthesia. Background ruled in sq. cm.)

THE RELATION BETWEEN URETER, VENOUS, AND ARTERIAL PRESSURES IN THE ISOLATED KIDNEY OF THE DOG

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AN increase in ureter pressure was believed by Ludwig [1861] to reduce the urine flow by reducing glomerular filtration without directly affecting tubular activity. Support for this view was obtained [Winton, 1931*a*] from comparison of a pair of isolated kidneys subjected to an equal reduction of urine flow, one by increasing the ureter pressure, and the other by reducing the arterial pressure. If the initial rates of flow and composition of the urine from the two kidneys were the same, the compositions, so far as urea and chloride content was concerned, were indistinguishable after reduction of the flow. This view of the action of ureter pressure was shown to have the important theoretical consequence that, if an agent produced a change in glomerular pressure but no change in tubular activity, that change in glomerular pressure could be measured in terms of the corresponding change in ureter pressure needed to keep the urine flow constant [Winton, 1931*d*].

An increase in venous pressure was also believed by Ludwig [1861] to reduce urine flow by reducing glomerular filtration without directly affecting tubular activity, mainly because the pressure in the venules was transmitted to the collecting tubules in close apposition to them. Support for this view was obtained [Winton, 1931*b*] from observations on isolated kidneys exactly similar to those mentioned above in connexion with ureter pressure. Equal reductions in urine flow produced in one of a pair of kidneys by a rise in venous pressure and in the other by a reduction in arterial pressure, produced changes in the urea and

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chloride contents of the urine which were not distinguishable. These two hypotheses concerning the actions of ureter and venous pressures, taken together with certain further evidence [Winton, 1931c], led to the formulation of the pressure law of the kidney, that the ratio of the changes in ureter pressure and venous pressure which produce equal changes in urine flow is equal to the ratio of the glomerular capillary pressure to the arterial pressure.

It was shown [Winton, 1937] that these theoretical developments required a re-examination of the experimental foundation of Ludwig's hypotheses, extending the evidence of the observations on isolated kidneys mentioned above to other constituents of the urine, and raising the level of accuracy, which involved uncertainties of about 10 % in the 1931 series. The experiments described below satisfy these requirements in so far as they include additional data on the creatinine concentration and conductivity of the urine; technically they differ from the earlier series mainly in being performed on the pump-lung-kidney preparation instead of on the less controllable heart-lung-kidney preparation, and in being performed on a single kidney submitted to simultaneous changes in arterial pressure and ureter pressure such that the urine flow remains constant, instead of on a pair of kidneys, one submitted to a change in arterial and the other to a change in ureter pressure. Considerably greater increases in ureter pressure could thus be studied without abolishing the urine flow, and the consequent changes in composition of the urine were correspondingly larger.

The new observations on venous pressure confirm the earlier ones in discovering no direct action on tubular activity. The increases in ureter pressure, however, produced changes in creatinine clearance somewhat different from the effects of arterial pressure, and this led to further experiments designed to estimate the error attributable to this factor in the pressure laws which, as mentioned above, were founded on the hypothesis that ureter pressure had no direct influence on tubular activity. A maximum value of this error can be expressed in terms of the difference between the ureter pressure which exactly counteracts the effect of a given increase in arterial pressure on the urine flow, and the ureter pressure which exactly counteracts the effect of the same arterial pressure on the glomerular filtration rate. In so far as the latter value can fairly be measured as the creatinine clearance, the experiments to be described show that this error in the pressure laws does not exceed 5-10 %.

METHODS

The perfusion technique and the chemical methods employed in these observations were the same as those described in earlier papers of this series [Eggleton, Pappenheimer & Winton, 1940*a*, *b*].

The main difficulty in securing accuracy in experiments on the relation between three variables, arterial pressure, ureter pressure, and urine flow, is due to the circumstance that the urine flow is much more sensitive to small changes in arterial pressure when the ureter pressure is raised than when it is at atmospheric pressure. Our experiments involved keeping the urine flow constant when arterial pressure and ureter pressure were both raised or both lowered, and in view of the drift in the properties of the isolated kidney, it was imperative to minimize the time during which pressure adjustments were made so as to restore the urine flow to its original value. Two points in the technique now employed have contributed to the improvement in the level of accuracy as compared with that previously achieved: (1) More accurate control of arterial pressure was secured in the pump-lung-kidney preparation than in the heart-lung-kidney preparation, and it was possible to hold this pressure steady within 0.5 mm. Hg during the critical periods of the experiment, a specially constructed mercury manometer being employed to secure accuracy in measurement of pressure; rather more effective smoothing in the perfusion circuit was secured, thus minimizing the uncertain effects of pulsatile pressure. (2) The use of a drop recorder [Winton, 1939], which records as the length of a vertical line on the kymograph the period of time taken for each group of five drops to fall, enabled the urine flow to be controlled quickly so as to reach and maintain an accurately steady value.

RESULTS

Ureter pressure

Observations on twelve pairs of samples of urine, secreted at the same rate but under different pressure conditions are shown in Table I, and the sequence of events in a single experiment of the kind from which the data in the table are abstracted is depicted in Fig. 1.

It is a feature of the natural history of the kidney after isolation that the creatinine and urea clearances drift gradually down, whereas the chloride clearance is apt to rise progressively after an initial period of a few hours during which it remains steady at a low value. Careful account has been taken of these disturbances in base-line in calculating the means

TABLE I. Excerpts of pump-lung-kidney experiments summarizing the data on the effects of simultaneous changes in ureter pressure and arterial pressure adjusted so as to produce no change in urine flow. Each value given is the mean obtained from two or three consecutive samples of urine

Experi- ment no.	Ureter pressure mm. Hg	Arterial pressure mm. Hg	Blood flow c.c./min.	Urine flow c.c./min.	Creatinine clearance c.c./min.	Urea clearance c.c./min.	Urine con- ductivity NaCl mM.	Percentage increase in			
								Urine flow	Creatinine clearance	Urea clearance	Conductivity of urine
38	0	131	83	0.323	7.20	2.65	28.2	+9.0	-21	-18.0	+ 2.5
	48	173	93	0.352	5.70	2.17	28.9				
	57	173	99	0.342	4.62	2.03	33.5	-0.3	-10	0	-13.0
37	0	105	110	0.343	5.13	2.03	37.9				
	0	127	76	0.247	4.75	2.92	48.7	-0.4	-16	-18.0	- 5.6
	37	160	84	0.246	3.98	2.38	46.0	-2.1	-22	-12.0	+27.0
	0	84	81	0.330	4.00	2.69	43.0				
	46	134	96	0.323	3.57	2.37	54.7	+5.1	+ 1	+ 0.5	-12.0
	50	134	108	0.335	3.46	2.00	71.7				
36	0	80	100	0.318	3.42	1.99	81.7	-5.6	-16	-11.0	—
	0	120	174	0.215	7.00	2.04	—				
	28	149	190	0.203	5.90	1.81	—				
34	10	116	125	0.428	10.60	3.94	22.1	-1.4	-28	- 5.0	+53.0
	37	162	135	0.422	7.60	3.75	33.7	-2.7	-29	-23.0	+57.0
	9	123	163	0.438	10.00	4.15	43.4				
32	27	173	163	0.426	7.08	3.20	68.0	+4.0	-14	- 8.0	- 8.0
	32	115	104	0.343	7.17	3.09	16.3	+0.6	- 7	+ 2.5	- 5.0
	55	146	114	0.357	6.13	2.85	15.0	-2.9	-24	-14.0	+47.0
	55	146	114	0.357	6.13	2.85	15.0				
	31	115	120	0.355	6.60	2.78	15.8	-6.7	-23	-10.0	-25.0
	31	115	120	0.355	6.60	2.78	15.8				
	78	175	135	0.345	5.02	2.40	23.2				
	78	175	135	0.345	5.02	2.40	23.2				
	19	116	143	0.368	6.50	2.66	31.1				
Mean of values obtained on raising ureter pressure								-0.3±1.6 (8)	-22.5±2.0 (8)	-13.5±2.1 (8)	+21.5±7.0 (8)
Mean of values obtained on lowering ureter pressure								-0.3±2.4 (4)	-10.0±5.0 (4)	- 2.0±2.8 (4)	-14.0±3.6 (4)
Mean								-0.3±1.3 (12)	-10.0±3.7 (12)	- 7.8±2.5 (12)	+ 3.7±5.4 (12)

of the changes in the clearances, etc., due to simultaneous increase in ureter pressure and arterial pressure under isorrheic conditions.

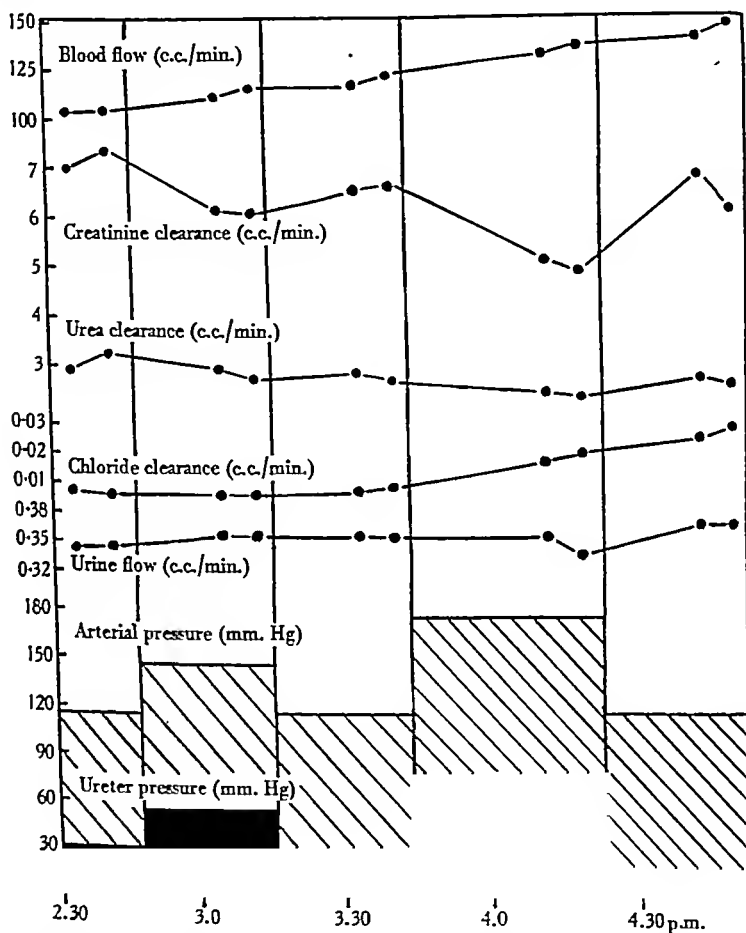


Fig. 1. Pump-lung-kidney experiment. Simultaneous changes in ureter pressure and arterial pressure adjusted so as to produce no change in urine flow, showing (1) the relation between equivalent ureter and arterial pressures, (2) the degree of steadiness of urine flow achieved, (3) no systematic effects on chloride clearance, (4) barely perceptible depression of urea clearance and substantial depression of creatinine clearance with increase in pressures. Kidney 20.5 g., perfusion begun 1.05 p.m., 1100 c.c. blood in circulation. Initial and final concentrations in serum: creatinine 33-27 mg., urea 131-132 mg., chloride 688-730 mg. (NaCl) per 100 c.c. Temp. 37.5° C.

It will be seen that there is a substantial decrease in creatinine clearance when the isolated kidney is subjected to a simultaneous increase in ureter pressure and arterial pressure without change in urine flow.

For an increase in ureter pressure averaging 39 mm. Hg, the decrease in creatinine clearance is 16 ± 3.7 %, and it is evident, therefore, that Ludwig's hypothesis that ureter pressure acts exclusively by reducing the glomerular filtration rate is untenable. The earlier observations [Winton, 1931*a*], which appeared to substantiate this hypothesis, were made only on the excretion of urea and chloride, when the ureter pressure was raised only about half as much as in the present series. Even with the greater increase in ureter pressure now made feasible by combining it with simultaneous increase in arterial pressure, the decrease in urea clearance is only 7.8 ± 2.5 %, a change which would have been too small to detect with confidence with the technique available in 1931. In a few experiments (e.g. Fig. 1) in which the chloride excretion was studied, and in the larger number (Table I) in which conductivity of the urine served as indication of electrolyte excretion, no systematic effect of increase in ureter pressure was detected. In this respect the present observations are in accord with those of the 1931 series.

The average values of the changes in ureter pressure and arterial pressure given in Table I are not strictly comparable, because in the first six observations the ureter pressure increase includes the intrarenal pressure. It has been shown [Winton, 1933] that if the ureter pressure be increased from atmospheric pressure in a series of small steps, no change either in urine flow or creatinine clearance occurs until a critical value, known as the intrarenal pressure, is exceeded. The intrarenal pressure acts essentially as if it were a pre-existing ureter pressure. Comparable values of the increase in ureter pressure and in the arterial pressure which, together, produce no change in urine flow, can be obtained either by subtracting the intrarenal pressure from the total ureter pressure, or by beginning the experiment with a ureter pressure exceeding the intrarenal pressure, and measuring the increase in ureter pressure associated with change in arterial pressure from this base-line.

The second of these methods was adopted in the second half of the series of observations depicted in Table I. In each experiment the ureter pressure was first raised to a low value which, however, sufficed to reduce the urine flow at that arterial pressure and was known, therefore, to exceed the intrarenal pressure. Subsequently the ureter pressure was further raised and the arterial pressure adjusted so as to prevent a change in urine flow. The latter changes in pressure are comparable because it has been shown [Winton, 1936] that an increase in arterial pressure does not substantially affect the intrarenal pressure, which remains, therefore, below the ureter pressure and without effect through-

out the experiment. From these observations only three mean values of the ratio of the change in ureter pressure to the change in arterial pressure which prevents change in urine flow can be obtained. They are 0.47, 0.76 and 0.89—average 0.71. Their significance will be discussed below.

Two values for the same ratio can be calculated with rather less confidence from the data in the first half of Table I, excluding the two observations on increase in pressures which were not balanced by observations on the corresponding decreases in pressures. In this calculation an average value for the intrarenal pressure of the isolated kidney must be assumed, and this value subtracted from the actual increase in ureter pressure. In comparable experiments the average intrarenal pressure is 10–15 mm. Hg [Winton, 1933], and if this value be adopted the ratios of the ureter pressure to its equivalent arterial pressure is 0.73–0.77 in the first, and 0.68–0.73 in the second experiment—average 0.73.

Venous pressure

Experiments, exactly comparable with those described above on ureter pressure, were performed on the effects of venous pressure on the isolated kidney. The results of one such experiment are depicted in Fig. 2. In Table II details of the analyses of thirteen pairs of samples of urine are given; in each pair the urine flow is about the same, but in one the arterial pressure is low and there is no obstruction to the venous outflow, and in the other both arterial and venous pressures are raised. It will be seen that the increase in pressures is not associated with a significant difference in creatinine clearance, in urea clearance, or in the conductivity of the urine. This provides additional and more accurate evidence confirming previous observations [Winton, 1931b] in support of Ludwig's hypothesis that venous obstruction reduces the urine flow exclusively by reducing glomerular filtration.

The mean venous and arterial pressure changes which appear from Table II to be equivalent with respect to change in urine flow are 50 and 41 mm. These pressures are, however, not strictly comparable because, as in the ureter pressure experiments, the intrarenal pressure should be deducted from the venous pressure if the change in venous pressure which is effective in changing the urine flow is to be obtained. If we assume the same average value for the intrarenal pressure as was adopted above, it is clear that there is no significant difference between the net change in venous pressure and the equivalent change in arterial pressure. The theoretical implications of this finding are discussed later.

TABLE II. Excerpts of pump-lung-kidney experiments summarizing the data on the effects of simultaneous changes in venous pressure and arterial pressure adjusted so as to produce no change in urine flow. Each value given is the mean obtained from two or three consecutive samples of urine

Experi- ment no.	Venous pressure mm. Hg	Arterial pressure mm. Hg	Blood flow c.c./min.	Urine flow c.c./min.	Creatinine clearance c.c./min.	Urea clearance c.c./min.	Urine con- ductivity NaCl mM.	Percentage change in			
								Urine flow	Creatinine clearance	Urea clearance	Conductivity of urine
43	0	95	44	0.331	2.95	—	—	- 7.0	- 30.0	—	—
	49	155	32	0.307	2.07	—	—				
	49	155	32	0.307	2.07	—	—	0	+ 9.0	—	—
41	0	124	45	0.308	1.90	—	—				
	0	116	123	0.237	5.30	—	13.9	- 2.5	- 10.0	—	+ 10.0
	50	173	140	0.231	4.77	—	16.5	+ 1.3	+ 17.0	—	- 16.0
39	0	165	170	0.228	4.07	—	19.6				
	0	87	108	0.274	10.02	4.20	18.0	- 4.4	- 23.0	- 14.0	+ 223.0
	37	129	95	0.262	7.70	3.60	58.2	+ 5.6	+ 10.0	+ 16.0	+ 58.0
36	0	101	136	0.248	7.00	3.10	36.8				
	0	108	174	0.214	9.75	2.51	—	+ 18.0	- 12.0	+ 10.0	—
	35	148	144	0.252	8.58	2.79	—	- 2.3	+ 10.0	+ 12.0	—
33	0	120	177	0.258	7.80	2.48	—				
	0	100	81	0.468	9.42	4.16	10.3	+ 1.9	- 8.0	- 5.5	+ 0.8
	50	130	80	0.477	8.65	3.93	11.0	+ 1.5	+ 17.0	+ 21.0	0
	0	93	77	0.470	7.37	3.25	11.0	- 5.1	- 7.7	- 8.9	+ 61.0
	0	93	77	0.470	7.37	3.25	11.0	+ 2.5	+ 1.8	+ 8.8	- 12.0
	79	160	85	0.446	6.80	2.96	17.7	- 0.5	- 9.1	- 5.9	+ 41.0
	79	160	85	0.446	6.80	2.96	17.7				
	0	105	90	0.435	6.68	2.72	20.1	+ 0.1 ± 3.2 (7)	- 14.3 ± 3.3 (7)	- 2.9 ± 4.0 (5)	+ 70.0 ± 40.0 (5)
	0	105	90	0.435	6.68	2.72	20.1	+ 1.4 ± 1.1 (6)	+ 10.8 ± 2.3 (6)	+ 14.5 ± 2.6 (4)	+ 30.0 ± 21.5 (4)
50	0	105	90	0.433	6.07	2.56	28.3	+ 0.7 ± 1.7 (13)	- 1.7 ± 2.8 (13)	+ 5.8 ± 3.3 (9)	+ 50.0 ± 31.0 (9)
	50	150	100	0.433	6.07	2.56	28.3				
Mean of values obtained on raising venous pressure											
Mean of values obtained on lowering venous pressure											
Mean											

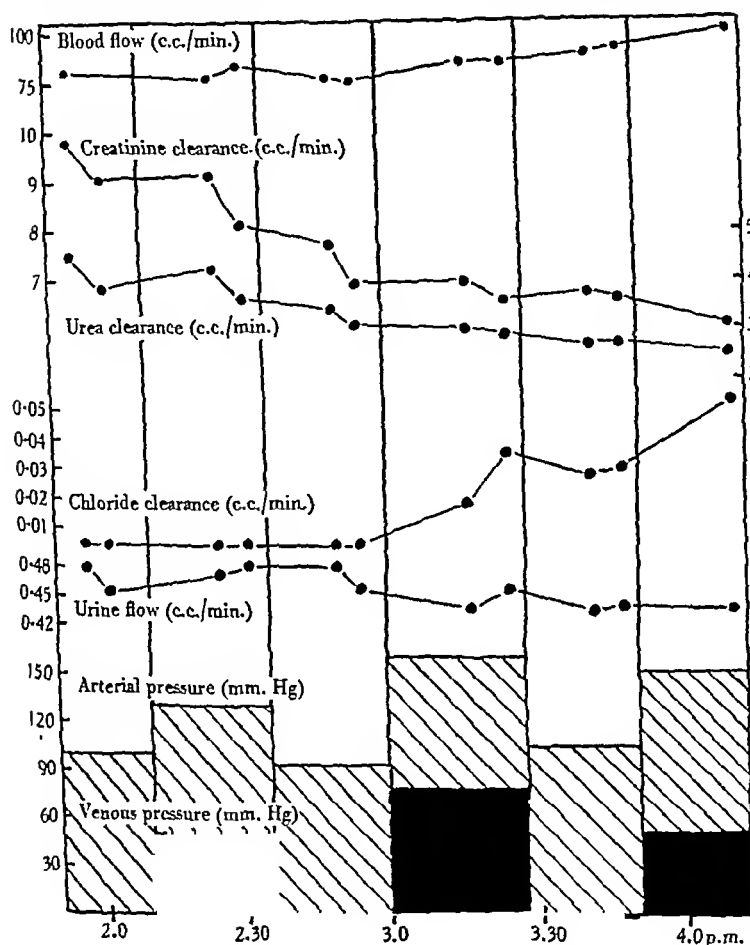


Fig. 2. Pump-lung-kidney experiment. Simultaneous changes in venous pressure and arterial pressure adjusted so as to produce no change in urine flow, showing (1) the relation between equivalent venous and arterial pressures when intrarenal pressure is ignored, (2) the degree of steadiness of urine flow achieved, (3) the absence of systematic changes in chloride, urea, and creatinine clearances with changes in pressure. Kidney 16 g., perfusion begun 12.40 p.m., 1050 c.c. of blood in circulation. Initial and final concentrations in serum: creatinine 43-33 mg., urea 126-126 mg., chloride 696-783 mg. (NaCl) per 100 c.c. Temp. 37.5° C.

Glomerular capillary pressure

The derivation of the pressure law of the kidney [Winton, 1931c] depended on the assumption that both ureter and venous pressures acted solely by reducing the rate of glomerular filtration. Since it has been

demonstrated above that this is true for venous pressure, but not exact true for ureter pressure, it would seem desirable to restate the law in the following more precise form: the ratio of the glomerular pressure to the arterial pressure is the same as the ratio of the increase in ureter pressure to that in the venous pressure when the latter pressures are so chosen that they produce the same changes in the rate of glomerular filtration and that they exceed the intrarenal pressure.

The pressure law is not as convenient experimentally in its new form in its original form, because it is more difficult to ensure an accurate match of rates of glomerular filtration than of urine flows. It seems desirable, therefore, to perform experiments designed to estimate the error in calculating the glomerular pressure from the original formula by comparing the increase in ureter pressure which would just neutralize the effect on the urine flow of a given rise in arterial pressure with that increase in ureter pressure which would just neutralize the effect on the glomerular filtration rate of the same rise in arterial pressure.

It was assumed, for this purpose, that if the creatinine clearance is the same under two conditions of unequal pressures, the glomerular filtration rate is also the same. The creatinine U/P ratio was kept well below the value, about 40, above which there may be deviation of the inulin clearance from the creatinine clearance [Shannon & Winton, 1944]. As it is impossible to know what the creatinine clearance is whilst the experiment is being performed, the procedure adopted was, in its simplest form, as follows: (1) Raise the ureter pressure to a value (P_1) which just slows the urine flow and so exceeds the intrarenal pressure. Let the urine flow and creatinine clearance as determined by subsequent analysis be termed U_1 and C_1 respectively. (2) Raise the ureter and arterial pressures, simultaneously adjusting the pressures so as to prevent any change from U_1 in the urine flow. The creatinine clearance C_2 will now be lower than C_1 . Let this ureter pressure be termed P_2 . (3) Keep the arterial pressure unchanged at its high value, diminish the ureter pressure to a value P_3 which is estimated to increase the creatinine clearance C_3 to a value slightly in excess of C_1 . (4) Restore ureter and arterial pressures to the values prevailing during stage 1 so as to check whether the base-line values of urine flow and creatinine clearance have remained reasonably steady during the intervening period.

The aim of the experiment is to discover the ureter pressure P_x which will produce the original clearance C_1 in the presence of the raised arterial pressure. This is readily calculated since the relation between ureter

TABLE III. Pump-lung-kidney experiment, showing the effects of simultaneous rise in ureter and arterial pressure without change in urino flow, and the effects of lowering the ureter pressure 11 % to produce a creatinine clearance somewhat above its initial value. Kidney weight 25 g., 950 c.c. blood in circulation, to which were added initially amounts to yield 25 mg. creatinine/100 c.c. and 100 mg. urea/100 c.c. Continuous infusion of 0.5 % creatinine and 1.5 % urea. Lungs connected to perfusion circuit at 11.0 a.m., kidney connected 12.55 p.m.

Time	Ureter pressure mm. Hg	Arterial pressure mm. Hg	Blood flow c.c./min.	Urino flow c.c./min.	Serum creatinine mg./100 c.c.	Creatinine clearance c.c./min.	Serum urea mg./100 c.c.	Urea clearance c.c./min.	Conductivity of urino NaCl m.M.
3.00	9.5	117	125	0.130	12.5	10.7	—	3.65	21.8
3.06	9.5	110	120	0.125	12.2	10.5	98	4.23	22.4
3.23	37.0	162	135	0.115	11.7	7.2	92	3.60	33.7
3.30	37.5	162	136	0.130	11.5	8.1	92	3.90	33.7
3.44	34.5	162	150	0.580	11.3	11.0	94	4.90	34.2
3.49	34.5	162	153	0.600	11.8	11.5	97	4.00	35.7
4.07	9.0	123	162	0.410	13.3	10.1	97	1.05	11.3
4.14	8.5	124	165	0.435	—	9.8	—	4.25	15.5

demonstrated above that this is true for venous pressure, but not exactly true for ureter pressure, it would seem desirable to restate the law in the following more precise form: the ratio of the glomerular pressure to the arterial pressure is the same as the ratio of the increase in ureter pressure to that in the venous pressure when the latter pressures are so chosen that they produce the same changes in the rate of glomerula filtration, and that they exceed the intrarenal pressure.

The pressure law is not as convenient experimentally in its new as in its original form, because it is more difficult to ensure an accurate match of rates of glomerular filtration than of urine flows. It seemed desirable, therefore, to perform experiments designed to estimate the error in calculating the glomerular pressure from the original formula, by comparing the increase in ureter pressure which would just neutralize the effect on the urine flow of a given rise in arterial pressure with that increase in ureter pressure which would just neutralize the effect on the glomerular filtration rate of the same rise in arterial pressure.

It was assumed, for this purpose, that if the creatinine clearance is the same under two conditions of unequal pressures, the glomerular filtration rate is also the same. The creatinine U/P ratio was kept well below the value, about 40, above which there may be deviation of the inulin clearance from the creatinine clearance [Shannon & Winton, 1940]. As it is impossible to know what the creatinine clearance is whilst the experiment is being performed, the procedure adopted was, in its simplest form, as follows: (1) Raise the ureter pressure to a value (P_1) which just slows the urine flow and so exceeds the intrarenal pressure. Let the urine flow and creatinine clearance as determined by subsequent analysis be termed U_1 and C_1 respectively. (2) Raise the ureter and arterial pressures, simultaneously adjusting the pressures so as to prevent a change from U_1 in the urine flow. The creatinine clearance C_2 will now be lower than C_1 . Let this ureter pressure be termed P_2 . (3) Keeping the arterial pressure unchanged at its high value, diminish the ureter pressure to a value P_3 which is estimated to increase the creatinine clearance C_3 to a value slightly in excess of C_1 . (4) Restore ureter and arterial pressures to the values prevailing during stage 1 so as to check whether the base-line values of urine flow and creatinine clearance have remained reasonably steady during the intervening period.

The aim of the experiment is to discover the ureter pressure P_x which will produce the original clearance C_1 in the presence of the raised arterial pressure. This is readily calculated since the relation between ureter

must be postulated, and the phenomena described could be explained by two classes of such changes. First, the increase in ureter pressure may inhibit tubular reabsorption of water; to prevent change in urine flow the pressure would, therefore, need to be high enough to reduce glomerular filtration, as indicated by the observed reduction in creatinine clearance. Secondly, increase in ureter pressure may increase leakage of fully formed or nearly fully formed urine from the distal segments of the tubules into the blood stream: such a leakage has been shown [Winton, 1935, 1937] to explain a number of properties of the kidney which otherwise fit rather awkwardly into our current conceptions of renal mechanism. This second explanation would imply that when the pressures are raised without change in urine flow, the observed reduction in U/P ratio is not due to a direct action on the tubules, but is of the same nature as the reduction always associated with increase in arterial pressure and the consequent increase in glomerular filtration rate. Unlike the reduction in U/P ratio due to simple rise in arterial pressure, the reduction due to the simultaneous isorrheic increase in arterial and ureter pressures fails to produce an increase in creatinine clearance or in urine flow because some of the urine is diverted from the distal tubules to the blood.

No direct evidence to distinguish between the two hypotheses is available. Both hypotheses imply that the increase of ureter pressure which neutralizes an increase in arterial pressure with respect to urine flow should be different from the value expected on the old view that ureter pressure was quite without influence on tubular function. It was shown above that the ureter pressures which just neutralized the effect of a given rise in arterial pressure with respect to urine flow on the one hand and to creatinine clearance on the other differed by 5-10 %. On the first hypothesis, involving inhibition of water reabsorption, therefore, the ureter pressure which prevents a change in glomerular filtration rate is 5-10 % lower than the rise in pressure measured (Table I) which prevented a change in urine flow. The second hypothesis, involving increased leakage from the tubules, on the other hand, would imply a ureter pressure higher than the previously expected value, and the order of this difference can be calculated from known properties of the isolated kidney.

Consider the reduction in the creatinine U/P ratio when arterial and ureter pressures are raised without change in urine flow. On the second hypothesis this is due to increased glomerular filtration, that is, the arterial pressure has been raised further than the pressure which would have just neutralized the effect of the ureter pressure on the glomerular

pressure and creatinine clearance is approximately linear at pressures considerably exceeding the intrarenal pressure. Consequently,

$$P_x = P_3 + \frac{(C_3 - C_1)(P_3 - P_2)}{(C_3 - C_2)}.$$

The values obtained in one such experiment are given in Table III, in this the increase in ureter pressure which prevents a change in creatinine clearance due to a given rise in arterial pressure is 6% lower than the increase in ureter pressure which prevents a change in urine flow due to the same change in arterial pressure.

The experiment depicted in Table III was exceptional because creatinine clearance was steadier than is usual in isolated kidney preparations. In further experiments the procedure had to be elaborated so as to enable us to measure the rate of decline of creatinine clearance with time and to allow for its influence. This could be done by interpolating another stage between the third and fourth stages described above. In this stage the ureter pressure was again raised to a value which at the same high arterial pressure, restored the urine flow to its original value U_1 . Instead of using the values C_1 , C_2 and P_2 in the final calculation, it was now possible, owing to the exactly symmetrical arrangement of the experiment and appropriate timing of the stages at equal intervals, to substitute the mean of the initial and final clearances for C_1 , the mean of the clearance in stage 2 and in the additional stage for C_2 , and to compare these means fairly with C_3 . Similarly the mean of the ureter pressures in stage 2 and the additional stage are comparable with ureter pressure in stage 3.

Experiments in which the urine flow in the final stage was substantially the same as that in the initial stage had to be discarded and external circumstances intervened to prevent our performing more than two successful experiments of this kind. The two additional values so obtained for the difference between the ureter pressures which maintain a given arterial pressure with respect to urine flow and to creatinine clearance were 5 and 10 %.

DISCUSSION

Ureter pressure

It has been established that there is a small but definite reduction in the creatinine clearance when ureter and arterial pressures are raised without change in urine flow. No change in glomerular filtration rate will, by itself, account for such a change in the relation between creatinine clearance and urine flow. Some change in the function of the tubules

0.55. In connexion with Table I, five values were calculated for the ratio of rise in ureter to that in arterial pressure which produced no change in urine flow, each value being the mean of a determination of the effect of rise and of the effect of fall in pressures, and therefore more reliable than the value derived from Table III. But it has been shown that the increase in ureter pressure which, combined with a given rise in arterial pressure, prevents change in creatinine clearance is 5-10 % lower than that which prevents change in urine flow. The values for the ratio of glomerular capillary pressure to the arterial pressure obtainable from Table I are, therefore, 0.44, 0.71, 0.83, 0.70, 0.66—mean 0.67 ± 0.07 .

If, however, as in the second hypothesis considered above, unchanged creatinine clearance does not denote unchanged glomerular filtration rate, and increased ureter pressure be supposed to increase leakage of fully formed urine from the tubules, the ratio of ureter to arterial pressure change which prevents a change in glomerular filtration rate is, as shown above, 1.06 ± 0.07 , implying a glomerular capillary pressure equal to or higher than the arterial pressure. This is impossible, for Ludwig's suggestion that the glomerular pressure should be unexpectedly high in virtue of the conversion of kinetic energy into hydrostatic pressure when the blood stream slows on entering the glomerular capillaries has been shown [Winton, 1931c] to account for a pressure increment of at most 2 mm. Hg. It is evident, therefore, that either the second hypothesis that ureteral obstruction increases leakage from the tubules is untrue, or there is some other major discrepancy in our analysis of the pressure relations in the kidney. This provides indirect evidence favouring the first hypothesis, that increase in ureter pressure inhibits reabsorption of water in the tubules.

The second pressure law of the kidney [Winton, 1931c] must, like the first, be modified by substituting constant glomerular filtration for constant urine flow. If it be allowed that equal changes in creatinine clearance denote equal changes in the rate of glomerular filtration, the second law may be restated in the form:

$$\frac{\text{Absolute glomerular pressure}}{\text{Absolute arterial pressure}} = \frac{\text{Increase in ureter pressure}}{\text{Increase in venous pressure}}, \quad (2)$$

when the increases are chosen so as to produce equal reductions in creatinine clearance if applied one at a time, and when the initial pressures exceed the intrarenal pressure.

In thirty comparisons of the equivalent ureter and venous pressures [Winton, 1931c, Table I] the ratio of the ureter to venous pressure was 0.69 ± 0.03 . The pressures then measured were equivalent with respect

filtration rate. Now in the kidney perfused under the relevant conditions, the effect of simple change in arterial pressure on the creatinine U/P ratio is to reduce it by about 1.25 % of the absolute U/P ratio per mm. Hg increase in arterial pressure. This is a mean value obtained from forty-four observations on twenty-five kidneys between 1931 and 1937, but not on the kidneys examined in the present series of experiments. The depression of the U/P ratio due to simultaneous rise in ureter and arterial pressure without change in urine flow is by definition the same as the depression in the creatinine clearances, shown in Table I to average 16 %. The arterial pressure in these observations at constant urine flow was, therefore, higher than that which would have kept the rate of glomerular filtration constant, on this hypothesis, by $16/1.25 = 13$ mm. Hg. If the individual increases in arterial pressure in Table I are reduced by an amount calculated in this way from the observed U/P ratios, we obtain the change in arterial pressure which would have prevented change in the glomerular filtration rate. On the second hypothesis, therefore, the ratio of the increase in ureter pressure (counted above the intrarenal pressure) to the corrected increase in arterial pressure averages 1.06 ± 0.07 .

Glomerular capillary pressure

In view of the fact now shown that the changes in creatinine clearance and urine flow are not exactly parallel when due to change in ureter and arterial pressure, the first pressure law of the kidney [Winton, 1931a] must be restated by substituting equal changes in the rate of glomerular filtration for equal changes in urine flow.

If it be allowed that a simultaneous increase in ureter and arterial pressure which produces no change in creatinine clearance involves no change in the rate of glomerular filtration, such an increase in ureter pressure should be practically equal to the increase in glomerular capillary pressure. If it be allowed, further, that a rise in arterial pressure produces a proportionate rise in glomerular pressure, then

$$\frac{\text{Absolute glomerular pressure}}{\text{Absolute arterial pressure}} = \frac{\text{Increase in ureter pressure}}{\text{Increase in arterial pressure}}, \quad (1)$$

when the increases are adjusted so as to prevent change in creatinine clearance if applied simultaneously, and when the initial ureter pressure exceeds the intrarenal pressure.

It has been shown, for example, in connexion with Table III that a rise in ureter pressure from 9.5 to 36 mm. combined with a rise in arterial pressure from 116 to 162 mm. would produce no change in creatinine clearance. Hence the ratio of the glomerular to the arterial pressure was

conditions of pressure, and no account was taken of intrarenal pressure. In such experiments, therefore, the increase in venous pressure should exceed the increase in arterial pressure by an amount about equal to the intrarenal pressure. The average values shown in Table II are 50 mm. for the rise in venous pressure, and 41 mm. for the rise in arterial pressure, a difference of 9 mm. which is a normal value for the intrarenal pressure of isolated kidneys under comparable conditions. As far as they go, therefore, the observed pressure equivalents accord well with the general theory of the pressure relations in the kidney which has previously been developed.

It is clear from the above algebraic analysis that no information about the glomerular capillary pressure can be derived from comparisons of equivalent venous and arterial pressures.

SUMMARY AND CONCLUSIONS

1. Simultaneous increase in arterial pressure and ureter pressure without change in urine flow reduces the creatinine clearance by $16 \pm 4\%$ and the urea clearance by $7.8 \pm 2.5\%$ in the isolated kidney of the dog. The chloride excretion and conductivity of the urine are unaffected (Fig. 1, Table I).

2. Simultaneous increase in arterial pressure and venous pressure without change in urine flow produces no significant changes in the creatinine and urea clearances or in the conductivity of the urine (Fig. 2, Table II).

3. The rise in ureter pressure which just neutralizes the effect of a given rise in arterial pressure on the creatinine clearance is 5-10 % lower than the rise in ureter pressure which just neutralizes the effect of the same arterial pressure on the urine flow.

4. The pressure laws of the kidney, with the reservations previously formulated [Winton, 1931*a*, *b*, *c*] should, therefore, be restated as follows: If ureter and venous pressures be measured from initial values which exceed the intrarenal pressure,

$$\frac{\text{Absolute glomerular pressure}}{\text{Absolute arterial pressure}} = \frac{\text{Increase in ureter pressure}}{\text{Increase in arterial pressure}}, \quad (1)$$

where the increases are applied simultaneously, and so chosen that there is no change in the glomerular filtration rate.

$$\frac{\text{Absolute glomerular pressure}}{\text{Absolute arterial pressure}} = \frac{\text{Increase in ureter pressure}}{\text{Increase in venous pressure}}, \quad (2)$$

where the increases are applied one at a time and so chosen that they produce no change in the glomerular filtration rate.

to urine flow, but it would appear from the present series of experiments that the ratio would be 5–10 % lower if it had been determined by reference to equal changes in creatinine clearance. The restated pressure law would, therefore, now yield a value of 0.64 ± 0.05 as the ratio of glomerular to arterial pressure in the isolated kidney.

The restated pressure laws are, however, subject to certain assumptions which have been critically considered in previous communications [Winton, 1931c, 1937] and the values for the glomerular pressure obtained from them can only be adopted tentatively until these assumptions have been further tested, and because no other method of estimating the glomerular pressure in the mammalian kidney is yet available.

Venous pressure

The present observations on the effects of venous pressure fully confirm those of the previous series, and support by the additional evidence in connexion with the creatinine clearance the theory of the action of venous pressure formulated by Winton [1931b]. The application of this theory to the present series of experiments may be summarized algebraically in the following form. In an experiment in which venous and arterial pressures are simultaneously raised and adjusted so as to prevent change in urine flow, let p_v and p_a be the increases in venous and arterial pressures respectively, and let the initial glomerular capillary pressure be the fraction α of the arterial pressure. Then the increase of pressures will produce a rise in the glomerular pressure of αp_a due to increase in arterial pressure and $(1 - \alpha) p_v$ due to the damming back of the blood by the increased venous pressure. The whole venous pressure is, however, transmitted to the contents of the distal tubules, and thus produces an increase in intracapsular pressure of the same value p_v since there is no change in the rate of flow of liquid down the tubules. Since the pressures are adjusted so as to prevent a change in the rate of glomerular filtration, the rise in intracapsular pressure must be the same as the rise in glomerular capillary pressure, and we may write:

$$p_v = \alpha p_a + (1 - \alpha) p_v,$$

or

$$p_v = p_a. \quad (3)$$

This equality in the equivalent changes in venous and arterial pressures presupposes that the venous pressure changes are measured from a baseline above the intrarenal pressure.

The experiments described above were primarily directed toward an exact comparison of the compositions of the urine secreted under unequal

EXPERIMENTAL CEREBRAL CONCUSSION

By D. DENNY-BROWN AND W. RITCHIE RUSSELL

*From The Laboratory of Physiology, Oxford.**(Received 19 June 1940)*

EXPERIMENTAL concussion is a condition well known from the early investigations of Kocher [1901], Duret [1920], Polis [1894] and, more recently, Miller [1927] to be associated with a cessation of respiration and rise of blood pressure, of duration corresponding to the intensity of the blow. Re-investigation of the phenomenon in cats under nembutal anaesthesia confirms its appearance in severe degree, and ability to result in death, without macroscopic lesions of the brain stem. It is further established that the phenomenon can be elicited in the decerebrate animal, and corresponds with a passing depression of all bulbar reflexes (corneal, pinna reflexes, etc.). The respiratory centre is the most sensitive to percussion. Acceleration in movement resulting from the blow is the essential factor in the stimulus, for if the head is prevented from moving when struck the phenomenon fails to occur. Momentary deformity of the skull, and stimulation of superficial structures, therefore appear to play no part. An instant acceleration of the head from zero to 23 feet per second (or reverse deceleration) is the minimal stimulus for the cat. It is slightly higher for the macaque monkey. Labyrinthine stimulation likewise appears to have slight if any part in the phenomenon, for it is obtained after section of both eighth nerves. Rise of intracranial pressure does not accompany the phenomenon, though it is possible under certain circumstances to reproduce a similar effect by a shock-like rise of intracranial pressure alone.

The nervous effect of a blow is thus considered to be due to the physical acceleration directly transmitted to each and every centre.

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Increase in venous pressure = Increase in arterial pressure (3)

where the increases simultaneously applied produce no change in glomerular filtration rate or in the urine flow.

5. If no change in creatinine clearance denotes no change in glomerular filtration rate, the application of the first law to the isolated kidney yields a value for the ratio of glomerular to arterial pressure of 0.67 ± 0.07 .

6. If equal changes in creatinine clearance denote equal changes in glomerular filtration rate, the application of the second law to the isolated kidney yields a value for the ratio of glomerular to arterial pressure of 0.64 ± 0.05 .

7. Experimental values of equivalent venous and arterial pressures (Table II) are in accord with the third law.

8. The hypothesis that increase in ureter pressure increases a leakage of fully formed urine from the tubules to the blood is examined quantitatively and shown to have consequences which are highly improbable. The main effect of ureter pressure is to reduce glomerular filtration, a subsidiary effect probably to inhibit water reabsorption in the tubules.

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increases with increasing concentrations. Histamine tends to increase the weight of NaCl absorbed and markedly augments the weight of glucose taken up at any initial concentration.

That the resistance offered to the secretion of HCl by hypertonic solutions of NaCl is greater than that opposed by glucose solution of corresponding strength may be related to the greater tendency of NaCl to be absorbed. When the permeability of the gastric mucosa has been modified by histamine, the rate of absorption of glucose approximates to that of NaCl.

AN IMPROVED ELECTRICALLY-HEATED
OPERATING BOARD FOR SMALL ANIMALS

BY ROLAND JOHN

*From the Cancer Laboratory, Department of Pathology,
University of Sheffield**(Received 3 July 1940)*

THE apparatus to be described has been very useful in performing aseptic operations on rats and mice. It consists (Fig. 1) of an operating board supported by a low pedestal on a turntable which is mounted at one end of a larger base board. The remainder of the base board serves as an instrument table. The whole unit may be set up on the end of a laboratory bench or on a small table of suitable height.

As the operating board can be rotated into any position to suit the convenience of the operator, it is unnecessary to provide means for securing the animal in more than one position on the board and only four cleats are required. At one end of the board there is an extension to take the animal's head and also to accommodate the anaesthetic mask. An oblong hole, covered with a sheet of zinc, in the central part of the board transmits the heat from below.

The pedestal is built up in the form of a box and contains three 15-watt lamps of the type used nowadays as electric-cooker pilot lamps. It has been found that one such lamp is sufficient to maintain a suitable temperature, the remaining two, which are separately controlled by a switch, being used to expedite the initial heating of the board. The switch and also a socket for the electric supply are mounted on the side of the pedestal beneath the head extension where they are easily accessible to the anaesthetist. The pedestal raises the operating board 4 in. above the level of the base board. This permits the use of operating sheets large enough to come well down over the sides of the operating board, so helping to maintain the sheets in position, but not so large as to foul the base board during movements of the turntable.

THE INFLUENCE OF HISTAMINE ON THE
ABSORPTION OF SODIUM SULPHATE
BY THE STOMACH

BY N. B. MYANT

From the University Laboratory of Physiology, Oxford

(Received 20 June 1940)

HYPERTONIC solutions of Na_2SO_4 in the stomach of the cat under chloralose anaesthesia offer much less resistance to the secretion of HCl as provoked by 1 mg. histamine subcutaneously than do solutions of NaCl (cf. Pratt [1940]). For example, 4% Na_2SO_4 permits the secretion of 189 mg. HCl , whilst 8% Na_2SO_4 allows 63 mg. to appear.

Loss of SO_4^{--} in the quiescent stomach cannot be observed when the concentration of the solution is 4% or less, though a little (1.4 g.) disappeared in 4 hr. from an 8% solution. However, the simultaneous administration of histamine causes the disappearance of 1.1 g. from a 4% solution in 4 hr., and as much as 5.8 g. from an 8% solution. These amounts are comparatively much less than the amounts absorbed in similar circumstances from solutions of glucose or NaCl , but they are sufficient to demonstrate that the favourable influence of histamine on the absorption of dissolved substances from the stomach is not confined to substances, such as glucose and NaCl , which are readily absorbed in the intestine, but extends to the divalent sulphate ion, which is not.

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The turntable consists of a brass disk, screwed to the under surface of the pedestal, pivoted to and bearing upon a square brass plate which is, in turn, screwed to the base board. The pivot bearing is formed by a length of brass tube which passes through the centre of the brass plate, in which it is sweated with solder, and which is further supported in a hole drilled through the wooden base. The tube projects above the upper

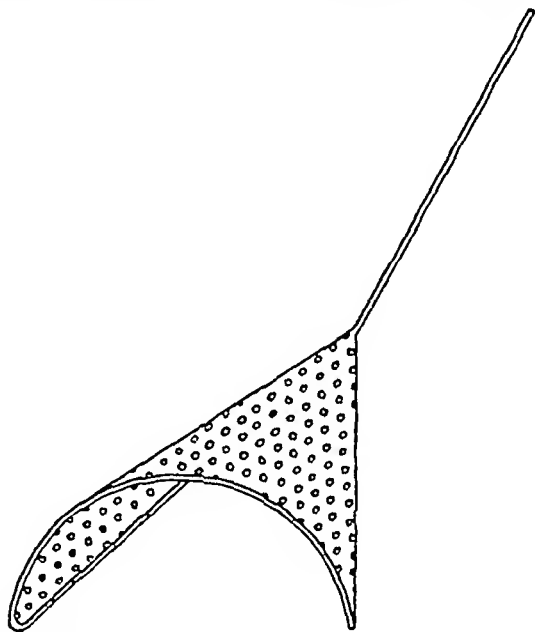


Fig. 2. The anaesthetic mask. Isometric projection, $\frac{1}{2}$ natural size.

surface of the plate so as just to pass through a hole drilled in the centre of the disk. The two parts of the bearing are held together by means of a $\frac{1}{4}$ in. brass bolt and castle-nut with split cotter. A spring washer under the head of the bolt provides a suitable bearing pressure between the two plates. This arrangement affords a smooth action combined with the necessary degree of stiffness.

The operating board, pedestal and base board are made of well-seasoned beech subsequently treated with several applications of linseed oil. The brass disk and plate are of 14-gauge sheet brass and brass screws are used throughout. The operating board, excluding the head extension, measures 10 × 10 in. and the instrument table 18 × 17 in. Other dimensions may be obtained from Fig. 1 which is an isometric drawing, $\frac{1}{2}$ natural size. A convenient size for the operating sheets is 20 × 16 in.

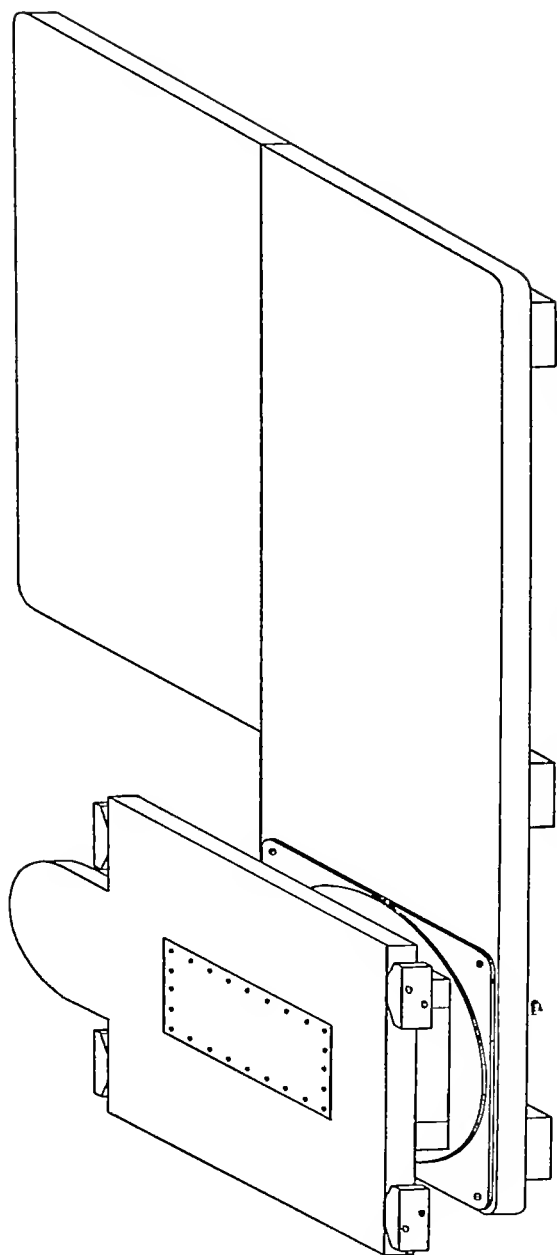


Fig. 1. The operating board unit. Isometric projection, $\frac{1}{2}$ natural size.

THE INFLUENCE OF THE SYMPATHETIC NERVOUS
SYSTEM ON CAPILLARY PERMEABILITY

BY D. ENGEL

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THE sympathetic nervous system has a great influence on the distribution of substances from the blood to the tissues by regulating the calibre of the blood vessels. It was the object of our experiments to show that the sympathetic plays another, possibly equally, important part in this distribution, by regulating the capillary permeability.

The importance of such a mechanism may be realized if one considers that no physiological function is possible without normal permeability of capillaries and cells in general. The intake of food, the excretion of end-products, every specific action of the protoplasm depends on normal permeability. It is governed in unicellular organisms chiefly by physical forces. In more highly developed animals conditions are much more complicated; published experimental evidence indicates that the sympathetic influences this fundamental function.

The literature on the subject was critically surveyed up to 1929 by Gellhorn [1929], who emphasized the desirability of investigating the conflicting ideas held by different workers. Since 1929 little progress has been made. It should suffice, therefore, to refer the reader to Gellhorn's monograph, and point out only the most important items from the literature. It was Asher & Jost [1914] who first ascribed a permeability increasing influence to the sympathetic nerves, and it was Asher's school that was mainly responsible for this idea. Asher's belief is based on the following observation: after unilateral upper cervical sympathetic ganglionectomy in the rabbit [Kajikawa, 1922], fluorescein injected into the peritoneal cavity appeared in the aqueous humour of the anterior chamber on the sympathetomized side later than on the normal side. Also the albumen content of the aqueous, after repeated puncture, was found lower, and the conjunctivitis induced by mustard oil less intense on the side of sympathectomy.

The anaesthetic mask used in conjunction with the board has also been very useful. It consists (Fig. 2) of a frame made of 14-gauge galvanized iron wire to which is soldered a piece of perforated sheet zinc. The zinc is covered with a single thickness of lint (not shown) which is secured by means of a few cotton sutures passed through the perforations. The semicircular opening at the wide end of the mask is 2.2 in. in diameter and this size is suitable for use with adult rats. Fig. 2 is an isometric drawing, $\frac{3}{4}$ natural size.

In use, a "mattress" consisting of one or two thicknesses of lint is interposed between the animal and the operating board and is carried forwards beneath the head so that the under edges of the anaesthetic mask also lie on it. This arrangement, by reducing air leaks, allows of easy induction and smooth maintenance of anaesthesia, at the same time reducing the consumption of anaesthetic to a minimum.

SUMMARY

The construction of an operating board unit for use with small animals such as rats or mice is described. The special features of the unit are a swivelling operating board which is electrically heated and the provision of adequate space for setting out instruments. An efficient anaesthetic mask is also described.

paradox pupillary dilatation or the adrenaline mydriasis. Linksz concludes that the sympathetic decreases, while the sympathectomy increases the capillary permeability, contrary to Asher. He draws his conclusions from experiments performed on the eyes of rabbits, but his statements are based more on speculation than substantiated evidence.

As is seen from this review, there is hardly any contradiction between the different authors as far as their experimental results are concerned, but there is an antagonism in their interpretation. The majority opinion is that the sympathetic increases, while sympathectomy decreases capillary permeability.

In all these former experiments the conclusions drawn were based only on the quantity of dye and other substances passing through the membranes studied; the control of blood flow was conspicuously neglected. The amount of a dye or any substance filtered through a living membrane depends on several factors, only one of them being the permeability of the membrane. The amount is greatly influenced by the concentration of the dye inside the capillaries, by the hydrostatic pressure across the membrane, the membrane area and the temperature. A dilatation of the capillaries due, for instance, to sympathectomy, will increase the filtering area, and thus the amount of dye passing will increase if the permeability per unit area remains the same. The concentration of the dye within the capillaries will also be influenced by a vaso-dilatation and consequently by the blood flow. It is to be expected that when the rate of supply of blood to a tissue is low, the blood passing through the tissue may become depleted of dye, so that the concentration difference across the capillary membrane falls off. When the rate of supply is high, this effect will be less pronounced, so that, other factors being equal, a larger amount of dye may pass through the capillary walls. No conclusions can, therefore, be drawn as to the permeability of a living membrane from the amount of substance filtered alone without taking into account the rate of blood flow.

METHODS

Our problem was to study the influence of the sympathetic nervous system on capillary permeability, while keeping a record of the blood flow. For this purpose a method had to be used which allows the measuring of permeability and blood flow simultaneously. The studies were carried out on the knee joint and the adjacent quadriceps muscle.

A substance was injected intravenously or intramuscularly and the knee joint was afterwards perfused. The substance appearing in the perfusate has to pass the composite membrane consisting of the capillary

vessel need be ligated; in the dog, however, one lumbar artery and vein require ligation. Retracting the vena cava medially there is no difficulty in the dog and cat in finding the lumbar sympathetic chain; some difficulty may, however, be encountered in a small rabbit where the right and left chains are so close together that they can easily be confused. After defining the nerve it is followed 3-4 cm. above the crus of the diaphragm, cut as high as possible, and then the rami communicantes of all lumbar and the first sacral ganglia are severed and the chain cut below the first sacral ganglia. There are four to six lumbar ganglia. The rami communicantes are always crossed by an artery and a vein; these vessels can be avoided as a rule; they need not be ligated in cats and rabbits. Bleeding stops on compression or muscle graft. The nerve removed is dried and pasted into the protocol for reference. The length of the whole chain removed is usually between 8-14 cm. The operation is performed under aseptic conditions, and the animals recover a few hours after operation.

Technique of perfusion

The apparatus used consisted of two identical syringes of 10 c.c. which were mounted firmly side by side, the pistons of which could be pushed forward simultaneously by a metal wheel so as to empty the syringes at identical rates. The wheel was fixed to the end of a threaded rod of square section and was propelled by the rotation of a pulley correspondingly threaded in its centre. The rod perforated also the two branches of a supporting pillar. On account of the square-shaped perforating holes the rod did not follow the pulley in its rotation, but was pushed forward. By regulating the speed of the motor to which the pulley was connected by a system of reducing gears, and by utilizing syringes of different bore, the rate of perfusion was variable over a wide range.

Ringer's solution from each syringe was led by rubber tubing to a needle of medium size inserted into each knee joint medial to the patella. The perfusion fluid was warmed by an electrically heated jacket in its passage along the rubber tube. After the joint cavity had been distended with 2-3 c.c. of fluid, a similar needle was inserted lateral to the patella. The joint cavity of a medium-sized cat or rabbit holds approximately 2-4 c.c.; the articular recess reaches about 1-2 cm. above the upper margin of the patella and, by palpating this edge, no difficulty is encountered in getting into the joint cavity. Great care must be taken to avoid bleeding in order to keep the articular barrier intact. Only blood-free perfusates were considered satisfactory. The fact that the

endothelium, the synovial cells and the thin layer connecting the two, the three being termed the articular barrier. The permeability of this barrier was studied previously [Engel, 1940]. By removing all the lumbar and the first sacral sympathetic ganglia, the knee joint—and with it the articular barrier—can be deprived of its sympathetic innervation completely. The non-operated side is available as a normal control.

Simultaneously with the perfusion, the blood supply of the two knee joints was measured by two thermocouples inserted into the lower quadriceps muscles. The temperature of a tissue is in direct proportion to its blood supply, other things being equal. On the other hand the amount of dye carried to the membrane to be tested also varies proportionately with the blood supply. From this it clearly follows that, other conditions being unchanged, the dye excretion, if changed at all, ought to change in the same sense as the temperature, i.e. rise when the temperature increases, fall when it decreases. A deviation from this rule would be a sure indication that there is an interference by another factor.

EXPERIMENTS

Fully grown cats, rabbits and dogs of both sexes were used. The whole lumbar sympathetic chain, the XIIth thoracic and in most experiments also the first sacral ganglion were removed on the right side under ether anaesthesia (see technique). After an interval varying from 2 to 25 days a dye, mostly Fuchsin S, was injected intramuscularly at a distance or intravenously and the two knee joints were perfused with Ringer's solution under nembutal, sodium amytal or chloralose anaesthesia. Great care was taken that the rate of perfusion should be equal on both sides. For this purpose an apparatus devised by Mr Condon was used (*v. infra*). The perfusates of the two joints were collected separately, and the dye penetrating into the joint and appearing in the perfusate was estimated by the colorimetric method. Before estimation a drop of acetic acid was added to the perfusates to regenerate the Fuchsin which becomes discoloured in an alkaline medium. Simultaneously with the perfusion the temperature of the two quadriceps muscles was taken by the thermoelectric method every 5 min.

Operative technique

Through a median hypogastric incision 15 cm. long reaching the symphysis, the right ureter is displaced medially and the peritoneum covering the right psoas muscle incised. In the cat and rabbit no blood

which is an essential part of the knee joint, is anatomically very closely related to the quadriceps muscle. Secondly, it is known that some differences exist between the vaso-motor innervation of the skin and muscles, but no differences are known or are likely to exist between muscles and other adjacent deep tissues. The quadriceps temperature was, therefore, taken as representing that of the adjacent synovial membrane.

Ten cats, five rabbits and six dogs were used in this series. In control experiments, without any preceding operation, in which the two normal sides were compared, the dye excretion was practically equal in nine cases out of thirteen; in the four remaining cases the ratio was 19 : 12, 5 : 8, 3 : 5, and 2 : 3. The latter inequalities were probably due to some pathological condition, possibly an unavoidable trauma before or during the experiment.

RESULTS

The vaso-motor innervation of the muscles varies considerably with the species, as we shall see later. The results obtained in cats, dogs and rabbits will, therefore, be discussed separately.

A. *Dogs.* Six animals were used in this series. The knee joints were perfused 2-5 days after the lumbo-sacral sympathectomy. The relation between dye excretion and quadriceps muscle temperature is shown in a typical case (94) in Fig. 1. Here the temperature taken every 5 min. for 2 hr. was constantly higher by about 2.5°C . on the right sympathectomized side, than on the left normal side. Still the dye excretion tested in three different portions was constantly lower on the warmer side. In the first period of observation the ratio of excretion of the two sides was about 1 : 2, in the second and third it was 1 : 4. This means that *the colder extremity excreted two to four times as much dye as the operated warmer side.* We see also that, though the temperature difference between right and left side remained practically constant during the whole experiment, the dye excretion dropped between the first and second period by about 50%. In short, there was *no conformity between temperature and dye excretion.*

The results of the other dogs are given in Table I. From this we see that, though the temperature differences between operated and normal side ranged between 2.5 and 5°C ., the nonconformity between dye excretion and muscle temperature was of the same nature as in dog 94. In four dogs the dye excretion was decreased on the operated side, while the temperature was higher. In one dog the contrary relation prevailed: though the operated side was colder, the dye excretion was higher. In all these five cases there was no conformity between temperature and

needles are actually in the joint cavity can be judged from the clear perfusate, from the absence of a para-articular oedema and from the first drop of synovial fluid. It is advisable, however, to ascertain the position of the needles at the end of the experiment. The perfusates were collected every 20–30 min., according to the need of the experiment. The rate of perfusion was about 20–30 c.c. per hour.

Special care was taken that the two extremities should be in the same position to avoid unequal muscle tone. The two extremities were strapped down identically, strangulation and other trauma being avoided as much as possible. The knees were shaved at the time of sympathectomy and not before the joint perfusion, possible fallacies due to irritation of the skin being thus obviated.

Control of temperature

Two copper-constantan thermocouples were used, each consisting of 30 S.W.G. (0.3 mm.) wire, the copper being enamelled. Each couple was hard soldered at the ends and inserted into a serum needle, the junction then being filed flat with the angle of the needle point. The fit of the wires into the needle was sufficiently firm to prevent movement. The control constant temperature junction was of similar material and inserted into a Dewar flask containing water of about 32° C. Connections were made to a galvanometer (H. Tinsley & Co.) of 33.5 Ω resistance with a period of 2 sec. for a deflexion of 1 cm.

The simple circuit consisted of the galvanometer, a throw-over switch and the thermocouple. By means of the switch the galvanometer could be connected to either of the two thermocouples.

The apparatus was carefully standardized by taking several readings at known junction temperature differences, and the two thermocouples compared with each other before and after each experiment. The scale could be read to $\frac{1}{3}$ mm. which corresponded to a temperature change of 1/33° C.

A thermocouple was inserted into each quadriceps muscle 3 cm. above the upper border of the patella, transversely to the long axis of the femur and close to its lateral side, in order to avoid the femoral artery. The position of the needles was changed during and after the experiment to compare the muscle temperatures at different spots. The temperature was recorded every 5 min. Throughout all experiments the vaso-motor innervation and blood supply of the synovial membrane was taken as being similar to those of the adjacent quadriceps muscle. This liberty was taken for the following considerations: first, the knee recess

dye excretion. Only in one dog (93) out of six was a higher dye excretion associated with a higher tissue temperature, and even in this single case some slight bleeding occurred into the joint, so that this result was not reliable.

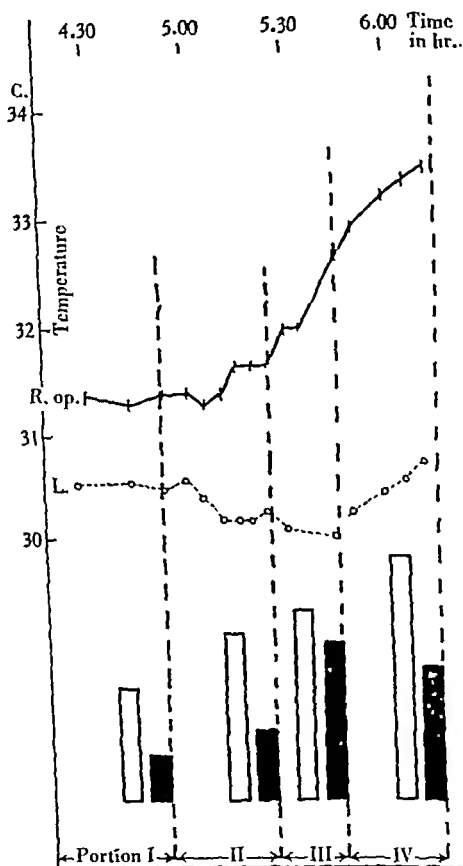


Fig. 2. Relation of dye excretion and muscle temperature in rabbit 105, 3 days after right sympathectomy. Dotted line: temperature of normal left leg. Solid line: temperature of denervated right leg. Black square: dye excretion (concentration) of right knee joint. White square: dye excretion of left knee joint. The four vertical lines divide the four periods in which the perfusates were collected (Port. I-IV). Note: high temperature on the right side is associated with low dye excretion (permeability); the reverse applies to the left leg. (In both legs the temperature is rising on account of the higher temperature of the heated table.)

B. *Rabbits*. Still more significant are the results obtained in rabbits because *their sympathetic nerves do not carry vaso-dilator fibres*, in contrast to those of dogs (Bülbring and Burn [1935]). A sympathectomy will,

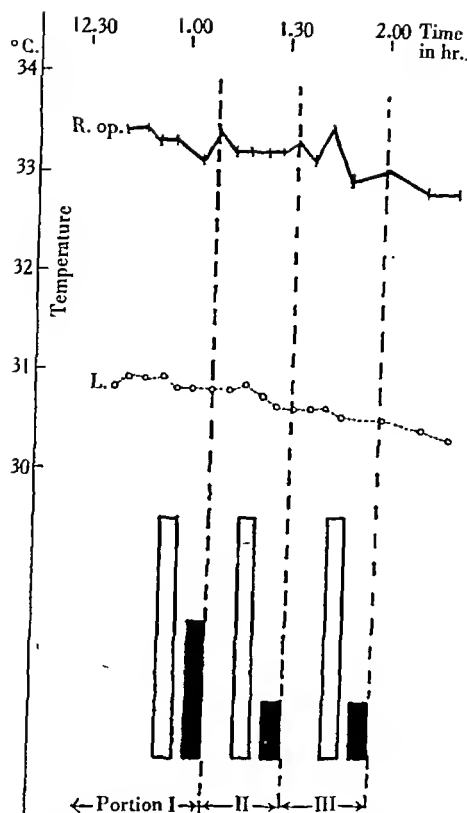


Fig. 1. Relation of dye excretion and muscle temperature in dog 94, 3 days after right sympathectomy. Dotted line: temperature of normal left side. Solid line: temperature of denervated right leg. Black square: dye excretion (concentration) of right knee joint. White square: dye excretion of left knee joint. The three vertical lines divide the three periods I-III, in which the perfusates were collected. Note: high temperature on the right side associated with low permeability, the reverse applies to the left side.

TABLE I. Relation of dye excretion—from the knee joint—to muscle temperature, after unilateral sympathectomy in dogs

No.	Days after op.	Excretion by op. side	Muscle temp. of op. side	Relation between two factors	Ratio of excretion between op. and norm. side	Remarks
91	3	Less	More (+5°)	No conformity	1 : 2.2	—
92	3	"	" (+3°)	"	1 : 3	—
94	3	"	" (+2.5°)	"	1 : 3.5	—
95	4	"	" (+1.5°)	"	Op. side: no secr. at all	—
96	5	More	Less (-0.5°)	"	2 : 1	Only 3 ganglia removed
93	2	"	More (+6°)	Conformity	2 : 1	—

op.=operated.

considerably on the right side, the relative dye excretion rose in proportion. The results of the other cats are shown in Table III.

The simultaneous measurement of the dye excretion from the joint cavity and that of the muscle temperature on the normal and sympathectomized side showed that, in the great majority of cats, there

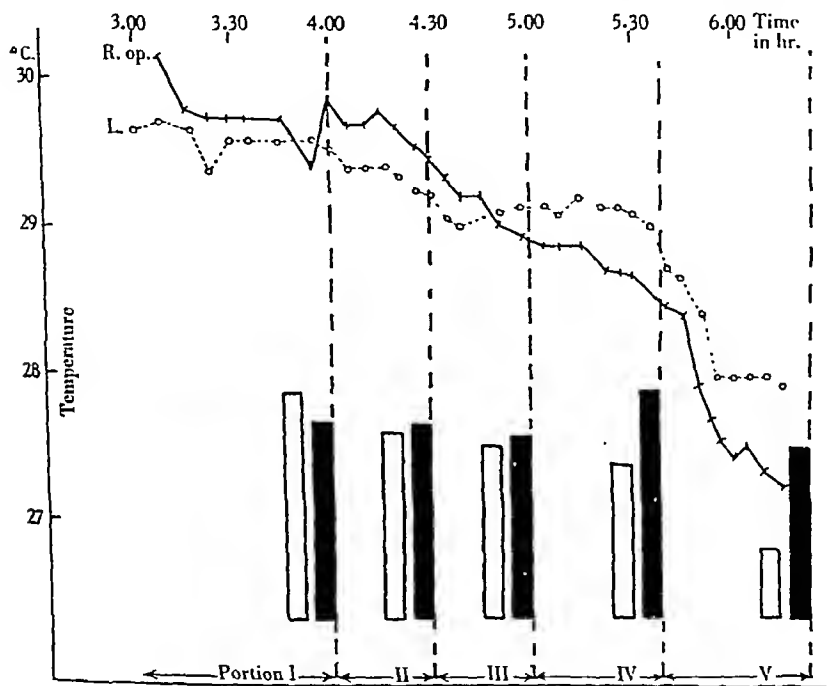


Fig. 3. Cat 84. Relation of dye excretion and muscle temperature 7 days after right sympathectomy. Dotted line: temperature of left, normal muscle. Solid line: temperature of denervated, right muscle. Black square: dye excretion of right knee joint. White square: dye excretion of left knee joint. Note: at the beginning of the experiment the temperature of the right side was higher and the dye excretion was lower; at the second half of the experiment the reverse relation prevailed.

was no conformity between these two factors. The dye excretion was in direct proportion to the adjacent tissue temperature only three times (A) out of ten animals, while in the other seven (B) the contrary was the case. In these seven cats, when the temperature was constantly higher on the sympathectomized side, the dye excretion was constantly lower (71), and *vice versa* (79). In most animals, however, there was a temperature fluctuation between the two sides during the experiment, so that at some periods the normal side, at others the operated side,

under comparison, might be affecting the results. It was, therefore, considered advisable to complete the investigations by a series of acute experiments, which should exclude such hypothetical errors.

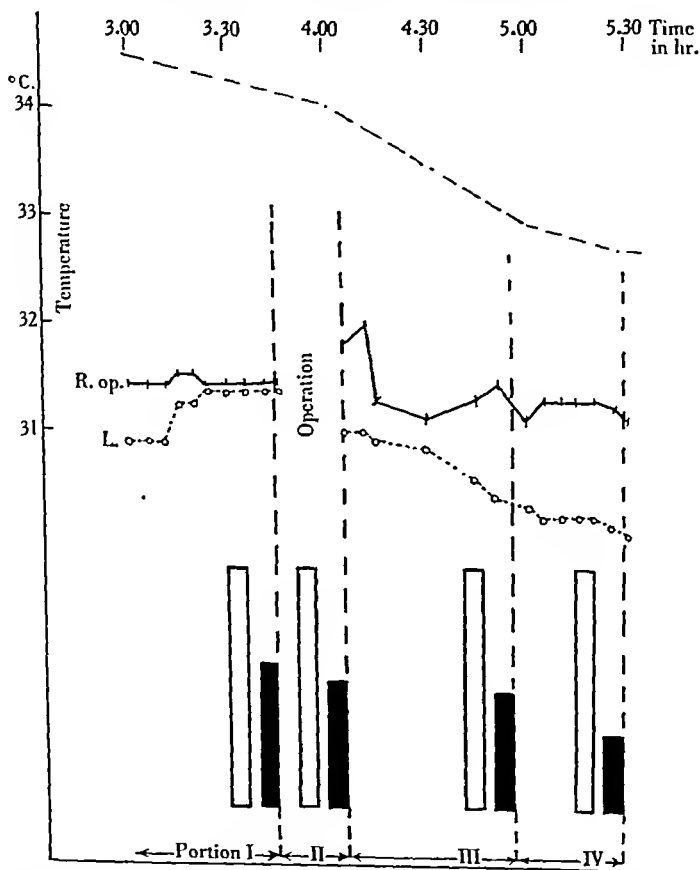


Fig. 4. Rabbit 115. Acute experiment. Relation of dye excretion and muscle temperature before and after right sympathectomy. Dotted line: temperature of normal left side. Solid line: temperature of operated right side. Black square: dye excretion of right knee joint. White square: dye excretion of left knee joint. Note: the dye excretion on the right side was lower in spite of the higher temperature, before operation. After operation the dye excretion was further reduced by 50 %, simultaneously with an increased temperature difference. Highest interrupted line: rectal temperature.

Acute experiments

In this series the perfusion of the two knee joints was started in a normal animal and the quadriceps temperature taken as in the previous series. One hour after the perfusion of the two normal joints was

TABLE III. Relation of dye excretion—from the knee joint—to muscle temperature after unilateral sympathectomy in cats

No.	Days after op.	Dye excretion by op. side	Muscle temp. of op. side	Relation between two factors	Ratio of excretion between op. and norm. side	Remarks
71	25	Less	More (+0.5°)	No conformity	8 : 9	Dorsal roots also cut
78	9	(1) More } (2) Less }	More (+1.0°)	"	9 : 8 7 : 9	—
79	14	More	Less (-1.3°)	"	6 : 5	—
84	7	(1) Less (2) More	(1) More (+0.5°) (2) Less (-0.5°)	"	7 : 8 2 : 1	—
85	6	(1) Less } (2) More } (3) Less }	More (+0.5°)	"	3 : 4 3 : 2	—
88	7	(1) Less (2) Equal	(1) More (+0.4°) (2) Less (-0.6°)	"	1 : 3 1 : 1	—
90	7	(1) Less } (2) More }	More (+0.5-1.0°)	"	2 : 3 2 : 1	—
83	5	Less	Less (-1.0°)	Conformity	7 : 10	—
86	7	More	More (+0.4°)	"	2 : 1	—
89	17	More	More (+0.6°)	"	7 : 5	—

(1), (2) and (3) mean the first, second, etc., period of the experiment.

was warmer. These cases revealed most clearly the antagonism between dye excretion and temperature, e.g. in two cats the *dye excretion of the operated extremity was higher while the temperature was low, and the excretion was lower while the temperature was high* (84 and 88).

Of the three cats of group A, two showed a constantly higher temperature along with a simultaneously increased dye excretion, and one a constantly lower temperature with a decreased dye excretion.

From the three series of experiments it becomes evident that in three different species the *dye excretion through the synovial membrane and temperature in the adjacent tissue did not follow a parallel course*. From twenty-one animals there was a direct proportion between the two factors only four times. In all of the other seventeen animals an increase in local tissue temperature, representing an increased blood supply, was associated with a decreased dye excretion and *vice versa*. This inverse proportion applied even to the same animal; two cats were observed in which the dye excretion went down, when the temperature went up, and *vice versa*. This relation of the two factors was so constant that it could not possibly be due to error or coincidence. The perfusion method is extremely sensitive, and it was thought that other factors, such as inflammation, trauma and individual differences between the two joints

animals were cats. From the eight animals with decreased dye excretion, six displayed a higher and only two a lower temperature. Thus, in this series of ten animals, in seven there was no conformity between muscle temperature and dye excretion.

If there was any doubt about the correctness of the interpretation of the results of the long-term experiments, these doubts should be dismissed by these corroborative findings.

Influence of the interval following sympathectomy, species and anatomy on the experimental results

A larger series of experiments (originally our preliminary experiments) was performed to ascertain what influence, if any, the length of the interval between sympathectomy and perfusion might have on the results. The number of the ganglia removed was also varied, in the same series, to localize anatomically if possible the site of the functional differences between the operated and normal side. The sex of the animals was recorded, in view of the fact that a permeability increasing hormone was found recently in the testicle.

It was further of interest to compare the behaviour of different species on account of their different vaso-motor innervation. It has been known since Dale's experiments [1906] that the sympathetic nerves carry not only vaso-constrictor but also vaso-dilator fibres. The distribution of these two types of fibres differs in different species [Bülbring & Burn, 1935, 1936]. In the rabbit and in the monkey there are no sympathetic vaso-dilators to the muscles; in the cat there are a few, while in the dog and in the hare they are numerous. A different dye excretion could, therefore, be expected in the various species. In the execution of the above experiments the same technique was used as in the previous ones, with the only modification that muscle temperature was not recorded.

From Tables V and VI it is evident that unilateral sympathectomy in cats and rabbits was followed in the majority of cases by a considerable decrease of dye excretion into the joint cavity, compared with the normal side. Out of twenty-five cats, fourteen had a decreased, nine had an increased excretion on the denervated side and two showed no change. While out of eight rabbits all except two displayed a decreased dye excretion on the operated side.¹

The results obtained were independent of the time that elapsed between operation and experiment, though this interval ranged from

¹ These figures may perhaps have a bigger significance than their statistical values indicate, if one considers the special vaso-motor innervation of cats, as will be discussed later.

started, the sympathetic chain was removed on one side, with the same technique and to the same extent as described before. The abdomen was closed temporarily with clamps. The operation lasted for about 15 min.; during this time the perfusion went on uninterrupted and the thermocouples remained in position. Thus, apart from the operation of unilateral sympathectomy, the conditions were constant throughout the experiment.

Results. One such experiment will be given in detail.

Experiment (rabbit 115). 15th Feb. 1940, a female rabbit of 2 kg. was used. Testing of each of the two thermocouples before and after the experiment showed that under identical conditions they gave identical readings.

At 1 p.m. Sod. amytal injected i.m.

1.50 p.m. The two thermo needles were inserted into the two quadriceps muscles close to the knee joints and the temperature read on both sides every 5 min. (see curve).

2.10 p.m. 20 c.c. of 2% Fuchsin S aq. solution injected into the pectoral muscle.

Between 3.45-4.05 p.m. The four lumbar and first sacral ganglia were removed on the right side, the left chain remaining intact. The perfusates were collected in four portions on each side and their dye content tested as usual. The temperature was measured at two other points in the muscle after the perfusion and the reading confirmed the correctness of the temperature indicated on the curve. The latter shows that the temperature, which was similar in the two muscles before the operation, was raised on the operated side by about 1° C. after the sympathectomy. Whereas the dye excretion of the right side, though lower from the beginning of the experiment, had decreased still further after the operation in spite of the higher temperature. The initial ratio of dye excretion 25 : 15 sank to a final ratio of 25 : 8—a decrease of about 50%.

As to the other acute experiments of the same nature, the results in six cats and four rabbits are shown in Table IV. Of these ten animals eight showed a decreased and only one an increased dye excretion after sympathectomy; one was uninfluenced by the operation. Both latter

TABLE IV. Acute experiments in cats and rabbits. Relation of dye excretion and muscle temperature after unilateral sympathectomy

Cat no.	Dye excretion by op. side	Muscle temp. of op. side	Relation between two factors	Ratio of excretion between op. and norm. side
107	Less	Unchanged	No conformity	1 : 3
108	More*	More (+1.0°)	Conformity	3 : 2
114	Less	Less	"	2 : 3
120	No change	"	No conformity	—
121	Less	More (+1.0°)	"	1 : 2
123	"	" (+1.0°)	"	1 : 3
Rabbit no.				
115	Less	More (+1.0°)	"	1 : 2
116	"	" (+1.5°)	"	1 : 3
117	"	Less (-0.5°)	Conformity	2 : 3
118	"	More (+1.5°)	No conformity	1 : 2, reversal later

* Excretion relatively lower, compared with previous period.

the lumbar ganglia or not. There were, however, no sufficient variations in the number of ganglia removed to allow a definite opinion as to the relation of anatomical localization and function. Three different narcotics, sodium amytal, nembutal and chloralose, did not influence the experimental findings.

The comparison of the excretion in the different species is of considerable interest. In those animals which have vaso-dilator and vaso-constrictor fibres in their sympathetic nerves (cats), one could suspect that if the sympathectomy is followed by a reduced dye excretion, it is because of an original prevalence of sympathetic vaso-dilator fibres. This possibility is certainly excluded in rabbits because of the absence of sympathetic vaso-dilators to the muscles. In these animals, possessing only vaso-constrictor fibres, sympathectomy is bound to give rise to pure vaso-dilatation. It should be anticipated, therefore, that the resulting improved blood supply would be followed by an increased dye excretion. But the contrary is the case; after the sympathectomy rabbits show a decreased dye excretion even in a higher percentage than the cats.

The same applies also to dogs (see Table I) in which, in spite of a double vaso-motor innervation, a sympathetic stimulation causes vaso-constriction, and consequently sympathectomy leads to a vaso-dilatation. Out of six dogs only two had an increased, and four a decreased dye excretion on the sympathectomized side. In one of the two dogs the result was dubious on account of a slight bleeding during the experiment.

The stimulation of the sympathetic chain at the level of the I-II lumbar ganglia gave inconstant results. In these experiments a faradic current with a coil distance of 12-14 cm. and non-polarizing electrodes were used.

Besides Fuchsin S, which proved very satisfactory and was used in most experiments, sodium thiocyanate and Echt-gelb were tried for control and gave identical results. The non-appearance of alkaline dyes in the perfusates, which is a normal finding, as discussed in a previous paper, was not influenced by sympathectomy; e.g. methylene blue, injected intravenously, was not excreted either by the normal or the sympathectomized side.

DISCUSSION

Reviewing all our experimental results we can state that in three different species—cats, dogs and rabbits—the penetration of a dye from the blood stream, through the articular barrier freed of its sympathetic innervation, was reduced, as compared with the normal side. These

TABLE V. Comparative dye excretion from the knee joint after unilateral sympathectomy in cats

No.	Days after op.	Excretion by op. side	Ratio of excretion between op. and norm. side	Which ganglia removed	Remarks
15	17	Less	1 : 2	I-IV L.	—
16	20	"	2 : 3	I-III L.	—
19	18	More	13 : 12	"	—
21	15	Less	1 : 2	I-IV L.	—
25	18	"	1 : 2	II-IV L. + I S.	—
30	30	More	2 : 1	I-IV L. + I S.	Repeated after 95 and 110 days
32	26	"	—	II-IV L. + I S.	—
36	11	"	6 : 5	"	—
38	71	Less	2 : 5	"	Repeated after 16 days
40	34	"	1 : 8	I-V L. + I S.	—
47	8	Equal	—	"	—
44	5	More	5 : 3	"	—
45	9	Less	—	I-V L.	—
69	4	"	3 : 4	I-IV L. + I S.	—
72	6	"	1 : 3	"	—
76	4	More	5 : 3	"	—
77	7	Equal	—	"	—
78	9	Less	3 : 4	"	—
79	14	More	5 : 3	"	—
83	5	Less	5 : 7	"	—
85	6	"	4 : 5	I-IV L.	—
86	6	More	2 : 1	"	—
88	7	Less	1 : 3	"	—
89	16	More	7 : 5	"	—
90	7	Less	1 : 2	"	—

TABLE VI. Comparative dye excretion from the knee joint after unilateral sympathectomy in rabbits

No.	Days after op.	Excretion by op. side	Ratio of excretion between op. and norm. side	Which ganglia removed	Remarks
53	10	Less	1 : 3	I-V L. + I S.	—
54	7	"	1 : 2	"	Reversal after 2 hr.
55	9	"	1 : 2	"	—
80	7	"	1 : 3	"	—
81	6	More	3 : 2	I-IV L. + I S.	—
97	3	Less-More	4 : 5-7 : 6	I-IV L.	—
104	6	More	5 : 2	II-IV L.	Op. incomplete
105	3	Less	1 : 2	I-V L. + I S.	—
39	46	"	—	I-IV L.	—

L. = lumbar ganglion.

S. = sacral ganglion.

3 to 86 days. In one cat, however, which showed a decreased dye excretion, as compared with the normal side, 71 days after the operation, the excretion became increased after a further interval of 15 days. No relationship between the sex and excretion was observed. Nor did it make any difference whether the first sacral ganglion was removed with

expect. From the experimental evidence one could conclude, moreover, that a compensatory system exists between vaso-motor activity on the one hand and capillary permeability on the other. Vaso-dilatation would seem to be accompanied by diminished permeability, the reverse effect being observed with vaso-constriction.

After sympathetic denervation three possibilities in regard to the effect on dye excretion can be considered: (1) if a balance is struck between the influence of vaso-dilatation and the factor affecting permeability, then there should be no change in the rate of dye excretion; (2) if capillary permeability is diminished to a degree outweighing the effect of vaso-dilatation, then the rate of dye excretion will be reduced; (3) if a reversed relation to that in (2) exists, the dye excretion will be increased. A changing ratio between these two factors could explain the results obtained in such an experiment as shown graphically in cat 84 (Fig. 3).

From the evidence presented in this paper it is not possible to decide whether two antagonistic factors exist which, respectively, increase and diminish capillary permeability, or whether only one such factor is operative. Nor is it clear whether the same or different factors control the calibre of the capillaries and permeability (simultaneously).

As to the nature of the "permeability factor" nothing definite can be said. From a teleological point of view, it would seem quite reasonable that when the capillaries are dilated some provision should be made to prevent extravasation of plasma constituents. Also from a purely anatomical standpoint, there is no contradiction between our experimental findings and the opinion generally held [Krogh, 1930], that the Rouget cells on the capillary wall are the contractile elements which provide the capillary tonus and are innervated by the sympathetic nerves; while the permeability depends on the state of the endothelium. The two factors, vaso-dilatation and permeability, would thus find their respective executive organs in two distinctly different cells. Krogh expressed the suspicion that a "permeability hormone" may exist. So far no opinion can be expressed as to the nature of our permeability factor or to its relation to the permeability hormone of the testicle and vitamin P. Attempts to extract this factor from tissues deprived of sympathetic innervation have so far failed.

One can, however, explain the antagonism between permeability and blood supply without assuming a hormonal factor. It is known that the capillary wall is highly sensitive to chemical changes, especially the oxygen supply. One can, therefore, assume that a lower oxygen supply as

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caused by a decreased blood flow, would affect the capillary endothelium and render it more permeable, while an increased blood flow would have a contrary effect. An antagonism between the two factors would thus automatically develop without having to postulate a hormone for explanation.

It was not the object of these investigations to find out whether the parasympathetic nervous system has a contrary effect to that of the sympathetic on permeability of cells. The existence of parasympathetic innervation to the leg is at least doubtful. There are, however, experiments known in another area, the lungs, which suggest an antagonistic effect of the vagi to the permeability increasing effect of the sympathetic nerves. v. Tschermak & Weiser [1933] produced an oedema of the lungs in rabbits, rats and guinea-pigs by cutting both vagi in the neck. The oedema developed within 48 hr. after operation. The authors explained their findings by an increase of the permeability of the capillaries, the lung epithelium and the tissue connecting the two. It would be premature to generalize on these findings with regard to other areas without further evidence. It seems also premature to draw practical conclusions from our experimental findings, especially if one considers the big differences existing between different species. But the poor results of sympathetic surgery [Simmons & Sheehan, 1939] are suggestive of the significance of the double role of sympathetic innervation.

Further experimental evidence in support of the above conclusions will be reported later.

SUMMARY

The influence of the sympathetic nervous system on capillary permeability was studied in dogs, cats and rabbits. The dye penetration from the blood through the synovial membrane was tested by perfusing two knee joints, one of which was deprived of its sympathetic nerve supply by unilateral lumbosacral sympathectomy. Changes in local blood flow were measured thermoelectrically.

It was found in the majority of both acute and long-term experiments that, in spite of marked vaso-dilatation, dye excretion was considerably reduced on the sympathectomized side.

This antagonism is explained by postulating a permeability factor which is under the influence of the sympathetic nervous system. The sympathetic activity increases, sympathectomy decreases capillary permeability. Such a permeability factor would counteract or balance the effect of the vaso-motor changes.

Grateful acknowledgement is made to the Medical Research Council for a grant towards the cost of the work and to Prof. A. J. Clark for his helpful criticism, suggestions and hospitality

these experiments have given no decisive support to the view that sympathetic nerves may influence the oxygen consumption of muscle. Thus Cannon *et al.* [1929] conclude that in the cat "...removal of the sympathetic chain does not reduce the metabolic rate more than 10%". It is noteworthy, however, that Nakamura [1921] tried the effects of electrical stimulation of the sympathetic chain on the oxygen consumption of the hindlimb muscles of the cat: under these conditions he observed a profound reduction in the oxygen consumption calculated as the product of the blood flow and A.V. O_2 -diff., a reduction for which he was unable to account.

An explanation of the results of Rein & Schneider and of Nakamura is, however, possible which would make unnecessary the conclusion that the sympathetic nerves exert a direct action in lowering the metabolism. If the action of the nerves were to divert blood into regions in which the oxygen consumption and surface area available for heat loss were low, then both the A.V. O_2 -diff. and the A.V. T.-diff. would be reduced. The product of blood flow and A.V. O_2 -diff. would be in this case only an apparent measure of the oxygen consumption which might in reality continue unchanged so long as adequate store of oxygen were available to the tissue deprived of a circulation.

In this paper it will be shown that electrical stimulation of the vasoconstrictor nerves to the isolated, perfused hindlimb or gastrocnemius muscle of the dog results in changes of blood flow and oxygen saturation similar to those which Rein & Schneider have described as occurring in the intact hindlimb during reflex vasoconstriction. Evidence will be presented that these changes are brought about by vascular rather than by metabolic means, and for this reason the product of A.V. O_2 -diff. and blood flow will be termed the "apparent oxygen consumption".

The isolated hindlimb provides a convenient preparation in which to investigate the phenomenon. The blood flow and oxygen saturation may be reliably measured and recorded over periods of several hours by the methods described by Kramer & Winton [1939] and the effects of changes in blood flow due to non-nervous sources such as variations in arterial pressure or in the composition of the blood may be studied and controlled.

METHODS

(1) *Perfusion.* The hindlimb or the gastrocnemius muscle alone was perfused at constant pressure with defibrinated blood from a pump-lung circulation. The apparatus and technique of perfusion were similar to

VASOCONSTRICTOR NERVES AND OXYGEN CONSUMPTION IN THE ISOLATED PERFUSED HINDLIMB MUSCLES OF THE DOG

BY J. R. PAPPENHEIMER

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(Received 22 July 1940)

REIN & SCHNEIDER [1937] have described experiments which indicate that reflex activity of the vasoconstrictor nerves to the hindlimb of the dog is accompanied by a closely associated reduction in the oxygen consumption of the limb. They have observed that during vasoconstriction elicited reflexly from the carotid sinus the arterio-venous difference in oxygen content (A.V. O_2 -diff.) may be diminished despite an unchanged or even a diminished blood flow through the limb. They found further that the arterio-venous temperature difference (A.V. T.-diff.) was diminished during the constriction, and that the magnitude of this diminution was of the order which might be expected from the calculated decrease in oxygen consumption. They have concluded that the sympathetic nerves exert a direct action in lowering the heat production and oxygen consumption of muscle.

The experiments of Rein & Schneider are in contrast to earlier investigations of a possible action of the sympathetic nervous system on metabolism. Previous investigations have been made with a view to obtaining evidence for or against a sympathetic control of tonus in skeletal muscle, and the presumption has been that if any change in metabolism should result from sympathetic activity it would be in the direction of an increase. The majority of early experiments were concerned with measuring the oxygen consumption or heat production of the whole animal or of muscle preparations before and after sympathetic denervation [Mansfeld & Lukács, 1915; Nakamura, 1921; Freund & Janssen, 1923; Newton, 1924; Cannon, Newton, Bright, Menkin & Moore, 1929]. The results of

oxygen consumption: the purified curare sometimes caused a slight vasodilatation, and when this occurred the oxygen content of the venous blood always increased also.

The alternating current was generated by interrupting a narrow beam of light focused on a photoelectric cell, with a rotating brass disk whose edges formed a sine wave in polar co-ordinates. The disk was mounted on ball bearings and driven through a gear box by a motor whose speed was recorded with an automobile speedometer. The amplified output of the photocell was approximately sinusoidal, and the apparatus was so arranged that the frequency could be conveniently varied from 2 to 300 c./sec.

EXPERIMENTAL

Fig. 1 shows the effects of stimulating the tibial nerve on the blood flow and oxygen content of venous blood in the isolated, curarized gastrocnemius muscle. The following description applies equally well, however, to the effects of stimulating the sciatic nerve in the whole hind-limb preparation. As the flow is diminished by vasoconstriction the venous blood becomes more saturated with oxygen and the A.V. O_2 -diff. is diminished. The apparent oxygen consumption is therefore reduced and more than in proportion to the blood flow. The magnitudes of these changes vary from one preparation to another, and in the same preparation with the frequency of the stimulus as will be shown below. With maximal stimulation a reduction in the apparent oxygen consumption to one-half the resting rate is usual, although a reduction to a quarter of the resting rate has been observed. When the reduction of blood flow is slight the reduction of apparent oxygen consumption may occur largely as a result of a diminished A.V. O_2 -diff. When the reduction of blood flow is great the A.V. O_2 -diff. may be unchanged or even slightly increased. In no case, however, has the apparent oxygen consumption failed to be substantially reduced.

The recovery of blood flow following release of the stimulus is accompanied by a decrease in the oxygen content of venous blood, and the magnitude of this decrease is such as to increase the apparent oxygen consumption above the initial value as seen in the analysis of the record. The apparent increase in oxygen use as measured by the area under the curve above the resting rate of 0.62 c.c. O_2 /min. was 0.27 c.c. O_2 as compared with the apparent decreased use of 0.52 c.c. O_2 which occurred during the stimulation. In the example shown, therefore, there occurred a net decrease in oxygen use of 0.25 c.c. O_2 as a result of stimulation.

those described by Kramer & Winton [1939] for the isolated kidney. In the hindlimb experiments the perfusion was made through the femoral artery and the venous blood collected from the femoral vein. Exclusion of the skin from the circulation did not affect the results to be described. In the single muscle preparations the hindlimb was skinned and the circulation confined as far as possible to the gastrocnemius muscle as described by Kramer & Quensel [1938]. The femur was then sawn through, and the muscle together with its nerve transferred to the perfusion circuit.

(2) *The A.V. O_2 -diff.* The oxygen saturation of arterial and venous blood was measured photoelectrically and recorded by the methods of Kramer & Winton [1939] with the modifications described by Eggleton, Pappenheimer & Winton [1940]. Kramer & Winton have indicated that the relative error in the measurement of A.V. O_2 -diff. by these methods may approach ± 0.02 vol. %. A more accurate appraisal of the absolute error involved in the measurement of oxygen combined with haemoglobin in blood of any given oxygen capacity is now available from a series of calibrations made with the manometric blood gas method (Van Slyke-Neill). The standard deviation of 97 gas analyses from the best straight lines in 31 calibration curves of the type shown by Kramer & Winton was ± 0.16 vol. %. The oxygen content of the blood as determined by gas analysis was corrected for dissolved oxygen on the assumption that the blood was in equilibrium with the 5 % CO_2 used to ventilate the lungs in the perfusion circuit. The curves covered the range 60–100 % saturation.

(3) *Blood flow.* The venous outflow was measured with a Gaddum Outflow Recorder calibrated at intervals during the experiment with a stopwatch and measuring cylinder. The excursion of the recorder was proportional to the blood flow within an error of about 3 %.

(4) *Arterio-venous temperature difference.* Single or multiple constantan-manganin junctions were inserted into the arterial and venous cannulae and the E.M.F. led to the same galvanometer-amplifier-recording system as that used for recording the oxygen saturation of the blood. The sensitivity of the system was generally adjusted by means of a shunt across the recording galvanometer so that a change of $0.1^\circ C$. produced an excursion of the recording milliammeter of 2 mm.

(5) *Stimulation.* The motor endings were paralysed completely by the addition of curarine or purified curare to the perfusing blood, and the mixed nerve supplying limb or muscle stimulated with induction shocks or with alternating current of controllable frequency. I am indebted to Dr H. King for giving me the curarine and advice concerning the purification of curare. Neither preparation had a detectable effect on the

Evidence concerning the mechanism of the phenomenon has been obtained from the following lines of enquiry:

I. Comparison of the effects of changes in blood flow produced by nerve stimulation with those produced by (a) change of perfusion pressure, (b) the action of adrenaline.

II. The effects of varying the frequency of stimulation.

III. Quantitative comparison of the apparent decrease in oxygen use with the apparent increase occurring during the recovery period.

IV. The action of ergotoxine.

V. The arterio-venous temperature difference.

I. (a) *Perfusion pressure*

The effects of changes in blood flow caused by changing the perfusion pressure have been observed in thirty-two hindlimbs and four gastrocnemius muscles. An increase in A.V. O_2 -diff. has never failed to follow a decrease in blood flow caused by a lowering of the pressure, and conversely a decreased A.V. O_2 -diff. has followed an increase in blood flow caused by a rise in pressure. A typical experiment is seen in Fig. 2. It is seen that the oxygen consumption of the gastrocnemius muscle is relatively unaffected by large changes in flow. In fifty-two experiments on eleven hindlimbs the mean diminution in oxygen consumption following a lowering of perfusion pressure has been 1.4 % per 10 c.c./min. reduction in blood flow over the range 50–200 c.c./min.

Two points concerning these observations should be made which will be discussed in greater detail elsewhere:

(i) The values of oxygen consumption given in Fig. 2 are equilibrium values taken not less than 2 min. after a change of pressure. Following a sudden increase of pressure there may be a transient increase of A.V. O_2 -diff. accompanied by a vasodilatation lasting some 20 sec.

(ii) As the blood flow is reduced below about 50 c.c./min. in the hindlimb the oxygen consumption may be considerably reduced although the A.V. O_2 -diff. continues to increase.

It is clear, however, that a reduction of blood flow is not in itself capable of producing the changes in apparent oxygen consumption which occur during stimulation of the vasoconstrictor nerves.

(b) *Adrenaline*

If the fall in apparent oxygen consumption during stimulation were due to a direct action of the sympathetic fibres on metabolism, it might be expected that a sympathomimetic substance such as adrenaline would

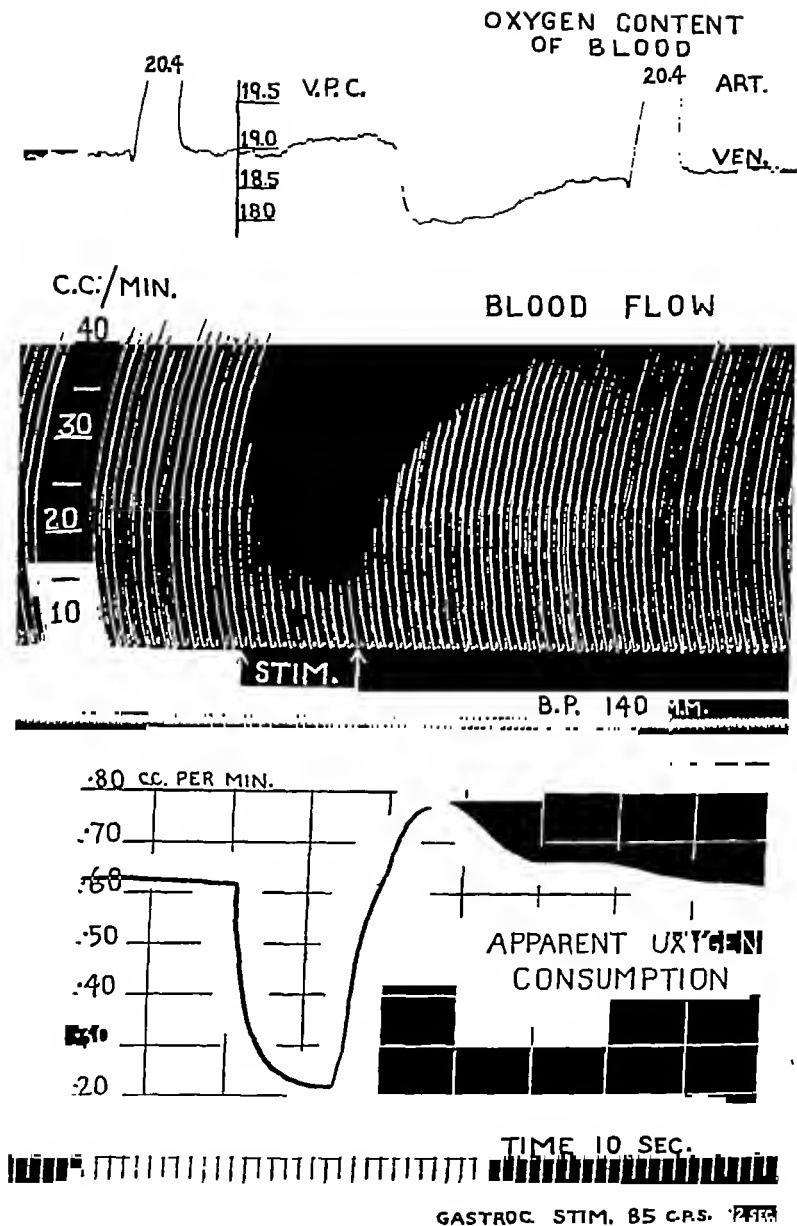


Fig. 1. The effects of stimulating the tibial nerve on the oxygen content of venous blood and on the blood flow in the isolated perfused gastrocnemius muscle. The arterial oxygen content was measured before and after the stimulation as indicated by the discontinuous sections of the venous oxygen record. Despite the reduction of blood flow the A.V. O_2 -diff. was diminished during the stimulation as indicated by the increase in the oxygen content of venous blood. In calculating the apparent oxygen consumption (product of blood flow and A.V. O_2 -diff.) corrections have been made for the curvatures of the recording levers and for the dead space between the recording unit and the vein.

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exert a similar action. If, on the other hand, the action of the nerves were to divert blood from localized parts of tissue then adrenaline might

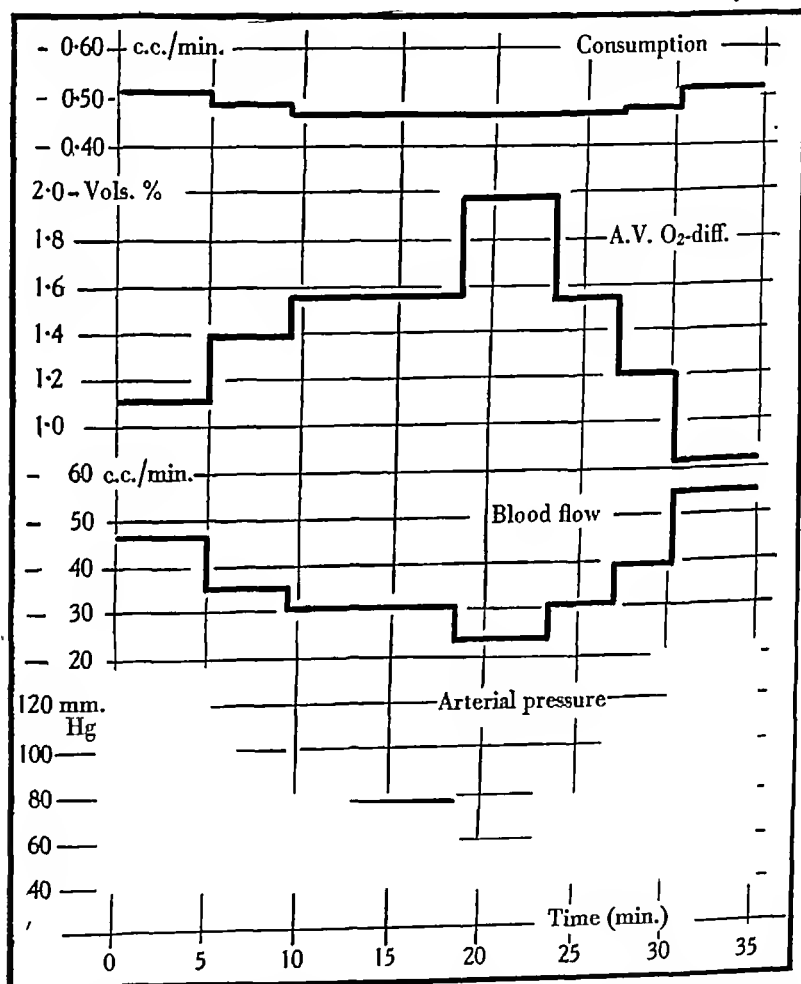


Fig. 2. The effects of changes in blood flow caused by changes of arterial pressure on the oxygen consumption of the isolated gastrocnemius muscle. The data are depicted diagrammatically in that only equilibrium values of blood flow and A.V. O₂-diff. are shown as explained in the text. The oxygen consumption at the lowest flow was 10% lower than that at the highest flow. Weight of muscle 46 g.

act differently, for in this case a more uniform vasoconstriction might be expected. .

Adrenaline in doses sufficient to cause a diminution of blood flow has without exception caused an increased A.V. O_2 -diff. With a moderate reduction of blood flow there may even be an increase in apparent oxygen consumption which returns to its initial value before the blood flow has recovered as shown in Fig. 3. Von Euler [1931] has reported a similar increase in oxygen consumption of the perfused hindlimb following

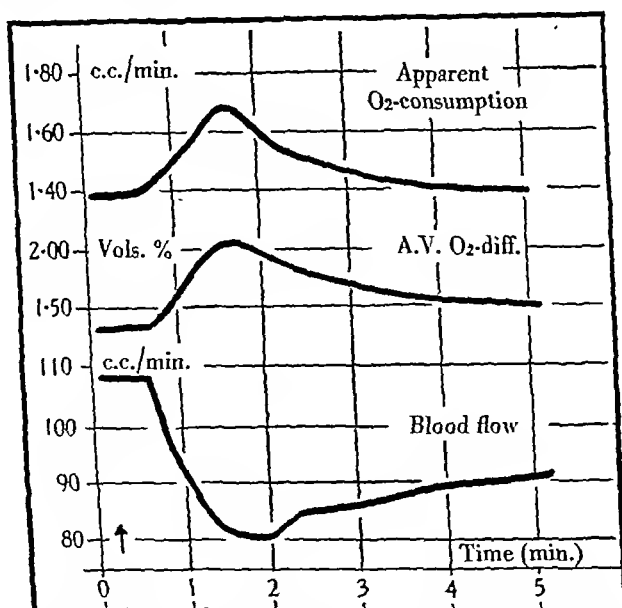


Fig. 3. The action of adrenaline on the blood flow, A.V. O_2 -diff., and apparent oxygen consumption of the isolated hindlimb. At the arrow 2.5 $\mu g.$ adrenaline were added to the perfusing blood (ca. 11.). The transient increase in apparent oxygen consumption has not been observed in all cases although the A.V. O_2 -diff. has never failed to increase.

small doses of adrenaline. We have not observed the increase in every case and do not feel that the data at present warrant a more detailed description.

It is clear, however, that although adrenaline and stimulation of the vasoconstrictor nerves have similar effects on the overall blood flow, they have widely different actions on the oxygen content of venous blood.

II. The effects of varying the frequency of stimulation

Valdecasas [1935] has shown that stimulation of the vasoconstrictor nerves to the hindlimb of the dog with alternating current of low frequency (18 c./sec.) results in a greater diminution of blood flow than

(ii) Intermediate frequencies, 15–100 c./sec. It is characteristic of this range that although the reduction of blood flow is of the same magnitude as that occurring during low-frequency stimulation, the recovery takes place gradually and may require several minutes to reach completion.

(iii) High frequencies, 100–300 c./sec. As the frequency becomes higher the magnitude of the reduction of blood flow becomes less: at frequencies of 300 c./sec. or above little or no response may be obtained, as found by Valdecasas.

If the effects of stimulation on the apparent oxygen consumption are due to vascular changes then the characteristic effects on the blood flow might be expected to be associated with characteristic effects on the oxygen saturation of venous blood. This is found to be the case. It is seen that the sudden partial recovery of blood flow following stimulation with 4.6 c./sec. was accompanied by a sudden release of unsaturated venous blood. At the intermediate frequency, on the other hand, the post-stimulation increase of apparent oxygen consumption was delayed along with the delayed recovery of blood flow. At 290 c./sec. the effects on both blood flow and apparent oxygen consumption were just perceptible.

The complex nature of the recovery at the lower frequencies could be explained by the existence of two or more sets of vasoconstrictor nerves having different excitabilities and different loci of action. Evidence in support of this hypothesis is shown in Fig. 5. In this experiment the frequency was changed during the stimulation from 150 to 4.4 c./sec. and then back to 150. The initial stimulation of 150 c./sec. caused a small diminution in both flow and A.V. O_2 -diff. When the frequency changed to 4.4 c./sec. there occurred a further diminution of apparent oxygen consumption. When the frequency returned to 150 c./sec., however, the blood flow recovered partially almost immediately, and the oxygen content of the venous blood fell rapidly despite the fact that the stimulus of 150 c./sec. continued.

The interpretation of these events in terms of an indirect vascular mechanism would be as follows:

During the initial period of stimulation at 150 c./sec., blood was diverted from certain localized parts of the limb through regions of lowered oxygen consumption. When the frequency changed to 4.4 c./sec. nerve fibres supplying new tissues were excited, and this resulted in a further diversion of blood and a further reduction in apparent oxygen consumption. But when the frequency returned to 150 c./sec. venous blood from regions which had been deprived of blood by the action of

does stimulation with higher frequencies (300–500 c./sec.). It seemed possible that if the effect of stimulating the sciatic nerve on the apparent oxygen consumption of the curarized limb were due to a direct nervous action, then such an action might be distinguished from vascular changes by varying the frequency of stimulation.

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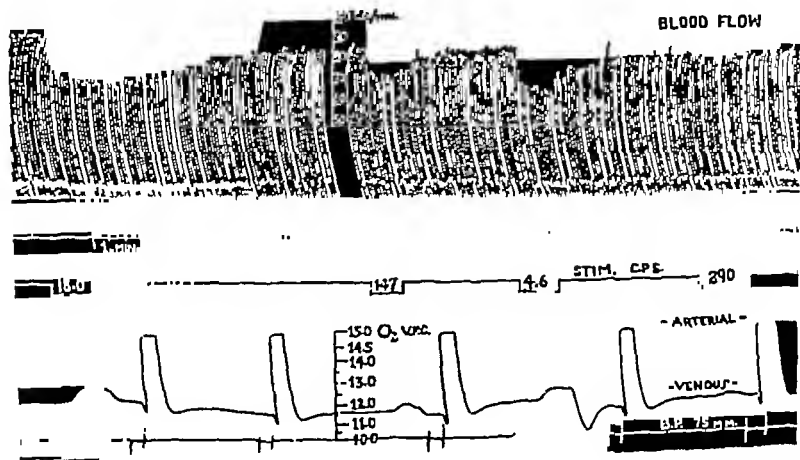


Fig. 4. The effects of stimulating the vasoconstrictor nerves with alternating current of different frequencies on the blood flow and oxygen content of venous blood in the isolated hindlimb. The sudden partial recovery of blood flow following stimulation with 4.6 c./sec. is accompanied by a sudden release of unsaturated venous blood. The gradual recovery of blood flow following stimulation with 18 c./sec. is accompanied by an equally slow change in the oxygen content of venous blood. Stimulation with 290 c./sec. produced a just perceptible effect on both blood flow and venous oxygen content.

confirmed on the isolated preparation. We have also investigated the effects of stimulating with frequencies below 18 c./sec.; this range has not previously been investigated.

Fig. 4 shows the effects of four successive stimulations of varying frequencies. Although the absolute values of the frequencies involved vary greatly from one preparation to another, their effects may, for descriptive purposes, be divided into three main groups:

(i) Low frequencies, 2–15 c./sec. It is characteristic of this range that when the stimulus is stopped the blood flow recovers partially within a few seconds. The remainder of the recovery occurs over a period of minutes.

(ii) Intermediate frequencies, 15–100 c./sec. It is characteristic of this range that although the reduction of blood flow is of the same magnitude as that occurring during low-frequency stimulation, the recovery takes place gradually and may require several minutes to reach completion.

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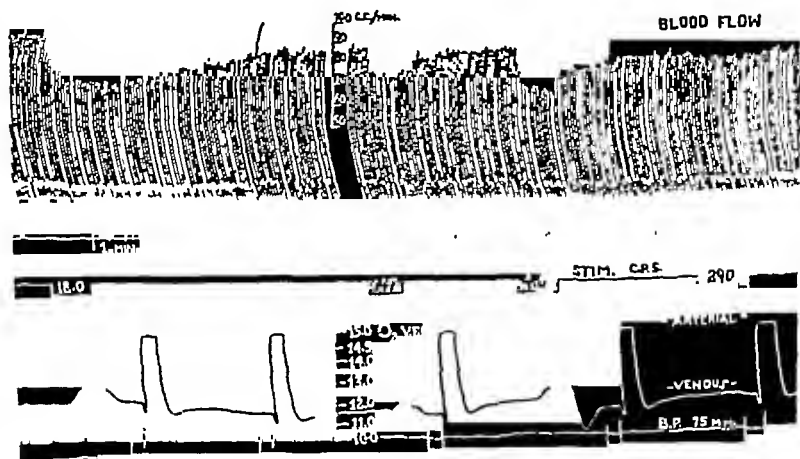


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During the initial period of stimulation at 150 c./sec., blood was diverted from certain localized parts of the limb through regions of lowered oxygen consumption. When the frequency changed to 4.4 c./sec. nerve fibres supplying new tissues were excited, and this resulted in a further diversion of blood and a further reduction in apparent oxygen consumption. But when the frequency returned to 150 c./sec. venous blood from regions which had been deprived of blood by the action of

fibres responding to low frequencies was released, so that the oxygen content of the venous blood diminished despite continued stimulation at high frequency.

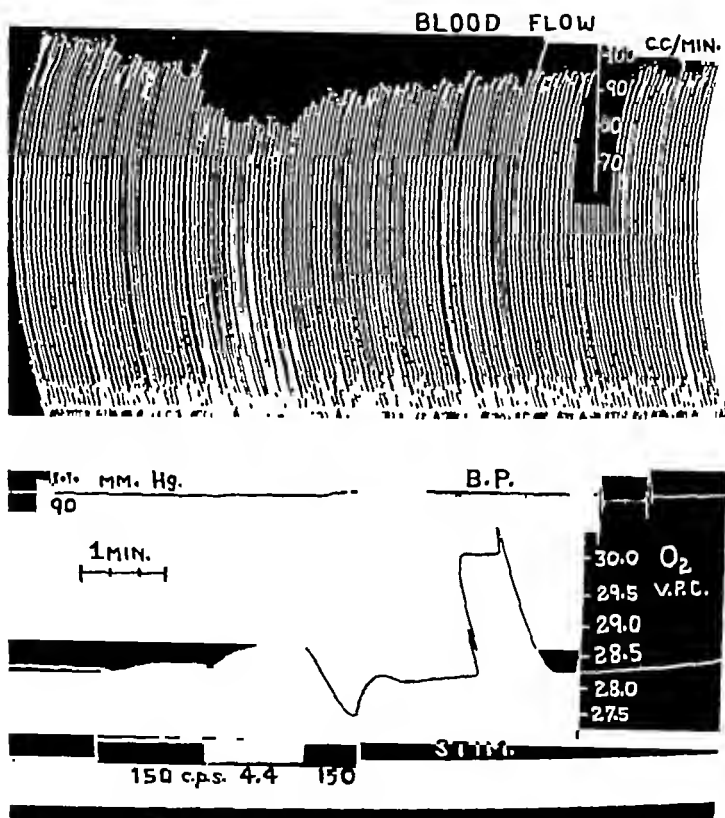


Fig. 5. The effects of sudden changes in frequency of stimulation of the vasoconstrictor nerves on the blood flow and A.V. O_2 -diff. of the isolated hindlimb. Following stimulation with 4.4 c./sec. the oxygen content of venous blood diminished rapidly despite continued stimulation with 150 c./sec.

The interpretation of these events in terms of a direct nervous action on the oxygen consumption would be difficult.

III. Quantitative relations

If the action of the vasoconstrictor nerves were to divert blood from parts of the muscle, it might be expected that the tissues deprived of a blood supply would continue to consume oxygen at the expense of oxygen

contained in the stagnated blood and combined with muscle haemoglobin. Provided that the store of oxygen were sufficient and that the rate of oxygen usage remained unchanged, the apparent increase in oxygen use

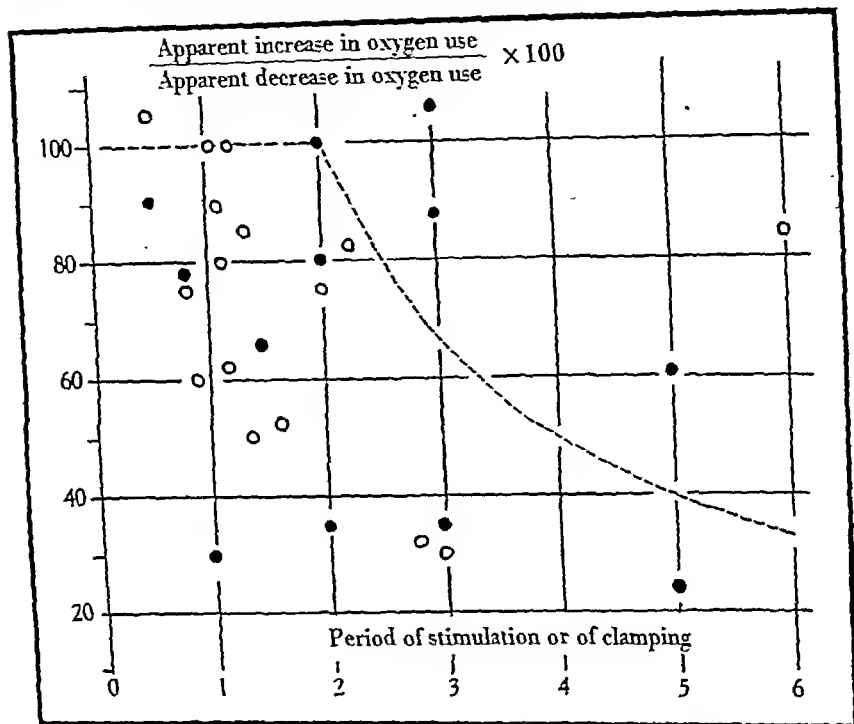


Fig. 6. The effects of clamping the circulation entirely for varying periods (black circles) or of stimulating the vasoconstrictor nerves for varying periods (open circles) on the ratio of the increase in apparent oxygen use following release of the clamp or of the stimulus to the apparent decrease in oxygen use which occurred during the period in which the stimulus or the clamp was applied. The broken line is the curve expected if the muscles continued to consume oxygen at an unchanged rate during the period of clamping or of stimulation until a 2 min. supply of oxygen were exhausted. Points to the right of this curve therefore indicate an increase in oxygen use greater than that obtainable from a 2 min. supply of oxygen stored in muscle. Compiled from observations on eight hindlimbs and two muscles. In no one experiment, however, were the effects of clamping compared with those of stimulation.

following release of the stimulation should exactly equal the apparent decrease which occurred during the stimulation. Millikan [1937] has shown that when the blood supply to the resting soleus muscle of the cat is clamped off, the muscle haemoglobin is reduced at the rate of approximately 1 %/sec. This would indicate that the supply of oxygen available

to resting muscles deprived of a circulation is sufficient to last less than 2 min.

In the example in Fig. 1 the stimulation lasted 92 sec., and the increase in apparent oxygen use as measured by the area under the curve above the resting rate of 0.62 c.c./min. was only 52 % of the apparent decrease which occurred during the stimulation. In order to investigate this question more fully we have compared the effects of clamping the circulation entirely for varying periods with those of stimulating the vasoconstrictor nerves for varying periods. The results are shown in Fig. 6. It is seen that the ratio of the increase in apparent oxygen use following release of the clamp or of the stimulus to the apparent decrease which occurred during the period of clamping or of stimulation bears no obvious relation to the period during which the stimulus or the clamp was applied. There is, moreover, little to distinguish the effects of stimulation from those of clamping in this respect. The broken line indicates the curve expected if the muscles continued consuming oxygen at an unchanged rate until a 2 min. supply of oxygen were exhausted. It may be noted that four of the points lie to the right of this curve, indicating that the increase in apparent oxygen use following the release of the clamp or stimulus was greater than that sufficient to supply the muscle for 2 min. The possibility exists, however, that the muscles may continue to metabolize anaerobically after the supply of oxygen has been exhausted, and that the debt so created may be repaid when the circulation is restored. Fenn [1930] and Rotta & Stannard [1939] have shown that this may be true of resting isolated frog's muscle which has been deprived of an oxygen supply.

Rein & Schneider [1937] reported that a considerable net decrease in oxygen use occurred as a result of reflex vasoconstriction, and considered the decrease as evidence for a direct sympathetic control of the metabolism. The results shown above indicate that a net decrease in oxygen use cannot be taken as evidence of direct nervous action, for clamping the circulation entirely for short periods may produce a similar net decrease.

IV. *The action of ergotoxine*

The results of stimulating at different frequencies have revealed a close association between vascular changes and changes in apparent oxygen consumption. If the effects on the apparent oxygen consumption are due to vasoconstriction then they should be abolished by the action of ergotoxine. Fig. 7 shows the effects of stimulating the nerve $\frac{1}{2}$ hr. after the addition of 5 mg. ergotoxine ethansulphonate (B.D.H.) to the per-

fusing blood (ca. 1 l.). Stimulation caused a vasodilatation accompanied by an initial fall in the oxygen content of venous blood followed by an increase which was of such a magnitude that the apparent oxygen consumption remained unchanged.

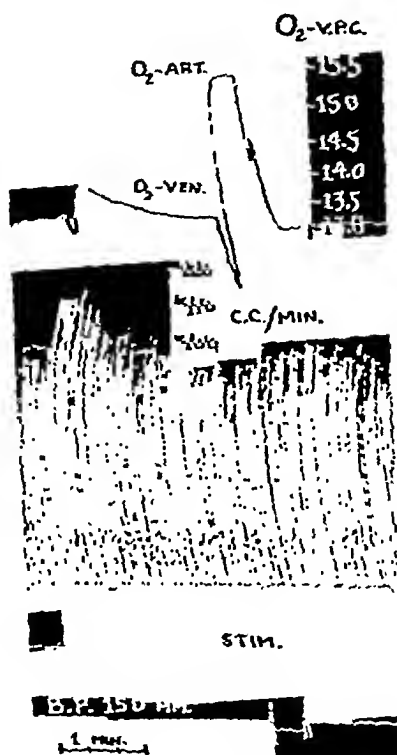


Fig. 7. The effects of stimulation following the addition of 5 mg. l. of ergotoxine ethan-sulphonate to the perfusing blood.

The initial fall in venous oxygen content is of interest, for it may be evoked by various agents causing vasodilatation and is presumably due to the release of small quantities of reduced blood from capillary fields which have been opened by the agent causing vasodilatation.

V. Arterio-venous temperature difference

At any given environmental temperature, the A.V. T.-diff. of the hindlimb is dependent on three factors: (a) the metabolism of the limb, (b) the area of cooling surface, (c) the velocity of flow. In the perfused

limb, blood in the venous cannula may be as much as 1.5°C . lower than that of blood in the arterial cannula. Under these conditions the heat liberated by metabolism is small compared to the heat loss from the

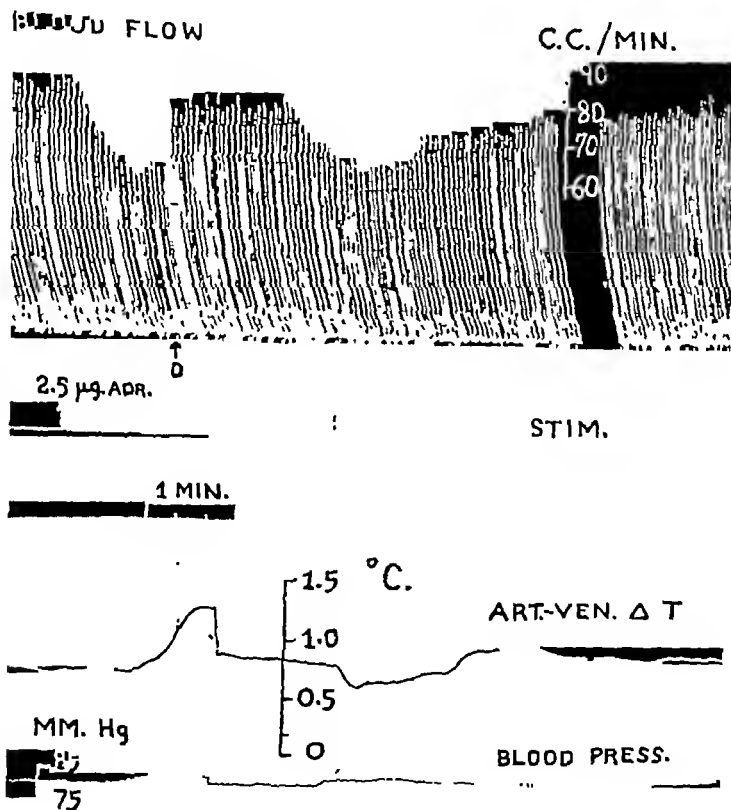


Fig 8. A comparison of the effects of approximately equal changes of blood flow caused by adrenaline and by electrical stimulation of the vasoconstrictor nerves on the A.V. T.-diff. of the isolated hindlimb. At *D* the record was stopped until recovery from the effects of adrenaline had taken place. The recording levers were not correctly aligned as may be seen from the record at *D*. The temperature changes actually occur within a few seconds of the changes in flow.

surface. Thus if the utilization of 1 c.c. of oxygen results in the liberation of 5 cal., then for a typical limb consuming 2.0 c.c. oxygen/min. at a blood flow of 100 c.c./min. the warming of venous blood due to metabolism should be only 0.1°C . If the blood flow were changed by mechanical or

pharmacological means the main factor determining the A.V. T.-diff. should be the velocity of flow since the area of cooling surface might be expected to remain constant. If, on the other hand, the reduction of blood flow involves the diversion of blood from large areas of tissue through regions of "lowered oxygen consumption" then alterations in the area of cooling surface might occur.

Fig. 8 shows a comparison of the effects of similar changes in blood flow caused by adrenaline and by stimulation of the vasoconstrictor nerves on the A.V. T.-diff. A reduction of blood flow caused by the action of adrenaline or by a lowering of the perfusion pressure has been found to result in an increased A.V. T.-diff., conversely an increase in blood flow caused by the action of histamine or by raising the perfusion pressure has been found to result in a decreased A.V. T.-diff. In all these cases the limb may be compared to a crude form of *thermostromuhr*, the A.V. T.-diff. varying inversely with the flow.

When the flow is reduced by stimulation of the vasoconstrictor nerves, however, the A.V. T.-diff. decreases instead of increasing. In the example shown in Fig. 8 the A.V. T.-diff. during constriction caused by nerve stimulation was 0.6°C . lower than during a similar constriction caused by the action of adrenaline. This difference is at least five times as great as could be accounted for by a change of metabolism. It seems reasonable, therefore, to conclude that the effect of stimulating the nerve is to divert blood through regions having a small surface available for the loss of heat.

DISCUSSION

It has been shown that the oxygen consumption of the perfused hindlimb or gastrocnemius muscle is apparently greatly reduced when the vasoconstrictor nerves are stimulated electrically. Two possible explanations of the effect have been considered, a direct action of the nerves on the metabolism and an indirect vascular mechanism.

While none of the evidence which has been obtained excludes the possibility that the action is a direct one, it renders such an hypothesis untenable unless further assumptions are made. It would thus be necessary to assume:

- (1) An explanation of the otherwise anomalous differences between the action of adrenaline and the action of the vasoconstrictor nerves
- (2) That when stimulation is stopped another mechanism is involved which increases the oxygen consumption

(3) That the mechanism assumed in (2) is affected by the frequency with which the nerves had been stimulated.

(4) That the nerves causing the changes in metabolism have the same electrical excitability as the vasoconstrictor nerves.

(5) That they are affected by ergotoxine in the same way as are vasoconstrictor nerves.

On the other hand, all the facts which have been observed admit of an explanation in terms of a vascular shunting mechanism without further assumption.

The question arises on the vascular hypothesis as to where the blood may be diverted and what tissues are deprived of a circulation during vasoconstriction. The regions through which blood may be diverted must, on the vascular hypothesis, consume oxygen at a rate lower than that of the muscle as a whole, for the apparent oxygen consumption is reduced more than in proportion to the reduction in blood flow. It has been shown that such regions have a reduced surface available for the loss of heat. These conditions would be fulfilled if the blood were diverted from capillary fields in the muscle tissue through arterio-venous anastomoses.

Perhaps the most widely used method of measuring the oxygen consumption of mammalian organs has involved the withdrawal of samples of venous and arterial blood and the calculation of the oxygen consumption as the product of the A.V. O_2 -diff. and the blood flow measured at the time of sampling. The inconstancy of the results obtained by the application of this method to resting mammalian muscle is well known. The variations have been emphasized by Barcroft [1934] and more recently by Looney & Freeman [1938] and by Holling [1939]. In this paper it has been shown that the oxygen consumption of the gastrocnemius muscle as measured by this method may vary fourfold within a minute as a result of stimulating the vasoconstrictor nerves (Fig. 1). In experiments on the innervated preparation in which the perfused limb has remained connected to the anaesthetized dog only through the sciatic nerve, we have found that large changes in the oxygen saturation of blood in the femoral vein may occur as a result of vasomotor activity in the dog. It would seem possible that such changes may be in part responsible for the variations in the apparent oxygen consumption which have been observed in previous investigations. The evidence presented in this paper suggests that the oxygen saturation of venous blood from resting muscle may be an indicator of the state of the intimate circulation within the muscle, rather than a measure of the metabolic rate.

SUMMARY

1. The oxygen consumption of the isolated perfused hindlimb or gastrocnemius muscle of the dog is relatively unaffected by changes in blood flow caused by changes of perfusion pressure. In eleven hindlimbs the mean diminution in oxygen consumption following a lowering of perfusion pressure was $1\frac{1}{2}\%$ per 10 c.c. min. reduction of blood flow over the range 200–50 c.c. min.

2. When the blood flow through the hindlimb or muscle is reduced by stimulation of the vasoconstrictor nerves the oxygen consumption calculated as the product of blood flow and arterio-venous oxygen difference (apparent oxygen consumption) is greatly reduced: the A.V. O_2 -diff. is usually diminished. The changes are similar to those which Rein & Schneider [1937] have described as occurring in the intact limb during reflex vasoconstriction.

3. When the blood flow is similarly reduced by the action of adrenaline the A.V. O_2 -diff. is increased and the apparent oxygen consumption may be increased also.

4. When stimulation of the vasoconstrictor nerves is stopped the apparent oxygen consumption is increased. This increase may be equal to or less than the diminution in apparent oxygen use occurring during the stimulation: no simple relation between the two quantities has been found.

5. The observations of Valdecasas [1935] that stimulation of the vasoconstrictor nerves with alternating current of medium frequencies (15–100 c. sec.) produces a greater reduction of blood flow than stimulation with high frequencies (100–300 c. sec.) are confirmed on the isolated preparation.

6. The effects of stimulation with frequencies lower than those which have been investigated previously (2–15 c. sec.) are described. It is shown that the blood flow recovers partially within a few seconds after such stimulation.

7. The characteristic effects on the blood flow of stimulating with different frequencies are accompanied by closely associated changes in apparent oxygen consumption.

8. The effects of stimulation on the apparent oxygen consumption are abolished by ergotoxine.

9. The arterio-venous temperature difference is increased when the blood flow is reduced by lowering the perfusion pressure or by the action of adrenaline. When a similar change in blood flow is caused by nerve

stimulation the A.V. T.-diff. is decreased. The changes observed are greater than can be accounted for by changes in metabolism.

10. The evidence suggests that the action of vasoconstrictor nerves is to divert blood from parts of the muscle through regions in which the oxygen consumption and surface available for heat loss are small. These regions may be arterio-venous anastomoses. It is, therefore, unnecessary to conclude that sympathetic nerves have a direct action in lowering the oxygen consumption of muscle.

I have pleasure in thanking Prof. Winton for his advice and help throughout the course of this work. This research was aided by a grant from the Faculty Research Fund, University of Pennsylvania.

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THE EFFECT OF ADRENALINE ON NERVE ACTION POTENTIALS

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It has been shown by Bülbring & Burn [1939] that in the perfused hind-leg of the dog, when the vascular tone is low, adrenaline can increase the muscular contraction by restoring the responsiveness of the preparation to stimulation of the motor roots. We have now investigated the effect of adrenaline on excitation and conduction in the sciatic nerve trunk of the cat with its natural circulation.

METHOD

Cats were used either anaesthetized with chloralose or decerebrated or spinal. The most constant preparation proved to be the spinal, eviscerated and adrenalectomized cat, although the phenomenon to be described was without exception obtainable in both the other preparations. The sciatic nerve was exposed in the gluteal region and divided above the point of entry of its blood supply from the gluteal vessels; the blood supply was thus carefully preserved. Below this a short stretch of nerve was freed for the application of Sherrington glass-shielded electrodes. The leg was held by bone drills through the femur, and the tendo Achillis was fixed to an isometric lever. The posterior tibial nerve was isolated in the lower half of the leg and arranged for leading off the action potentials with silver wires and thread loops. The femoral, the lateral cutaneous nerve of the thigh, and the external peroneal and hamstring branches of the sciatic nerve were cut. For repetitive discharge a neon stimulator was used and with high frequencies of stimulation a rotating commutator was put across the output to permit stimulation for 1/15th sec., three times per sec. A Keith Lucas pendulum and coreless induction coils were used for single shocks. To minimize the effect of changes in nerve resistance a resistance of 50,000 Ω was connected in series with the

electrodes. The recording apparatus consisted of a push-pull input stage [Toennies, 1938] with three further stages of R.C. coupled amplification and a cathode-ray oscillograph. Most of the work was done with a fixed plate camera and a linear time base. For the moving paper camera used in the later experiments we are deeply indebted to Dr E. H. J. Schuster.

All preparations were left for 1 hr. before recording. The thresholds could be relied on to stay steady for 3-4 hr. The intra-arterial injections of adrenaline were made through a cannula in the iliac artery of the opposite side pointing towards the aorta.

RESULTS

As Gasser & Grundfest [1939] have stated, the sciatic nerve action potential in the cat usually shows fused α and β spikes, which can be referred to as the $\alpha\beta$ spike, and a δ spike. Under various conditions of stimulation and with long conduction distances the α and β spikes may be separated (Fig. 3).

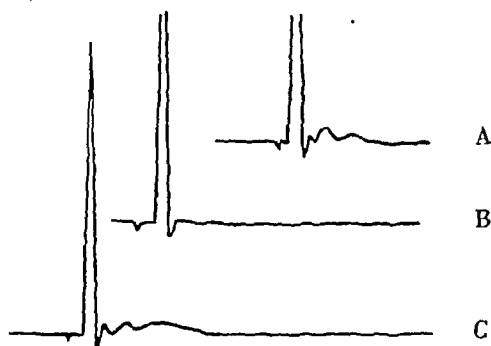


Fig. 1. Decerebrated cat. Action potentials set up by maximal stimuli 20 per sec. A, before; B, 50 sec. after 25 μ g. adrenaline; C, 2 min. later. Time: 1 d.v. = 10 msec.; 0.66 mV. = 1 cm.

The spike potentials from α fibres produced by maximal stimuli at any frequency up to 450 per sec. were completely unaffected by injection of any dose of adrenaline from 5 to 25 μ g. The δ spikes produced by maximal stimulation once per sec. or less were also unaffected by adrenaline. At frequencies of 5-20 per sec. the δ spike height fell rapidly at first to a steady level. Injection of adrenaline further reduced or abolished the δ spikes within 45 sec., and they recovered within 2-3 min. (Fig. 1). The maximal diminution coincided with the maximal rise in blood pressure

and passed off together with it. This effect of adrenaline on the δ spikes could be reproduced by occlusion of the aorta for 1 min. after which recovery occurred within 1 min. (Fig. 2). In our experiments fatigue of δ fibres occurred so rapidly with maximal stimuli at rates above 20 per sec., even when the blood supply to the nerve was undisturbed, that no observations on the effect of adrenaline during higher rates of stimulation were made.

With submaximal stimulation at frequencies from 1 to 250 per sec. intra-arterial injection of adrenaline in doses from 5 to 25 μ g. increased the $\alpha\beta$ spike by an average of 100% (Fig. 3). The amount of increase was not directly related to the dose of adrenaline, the requirement of which

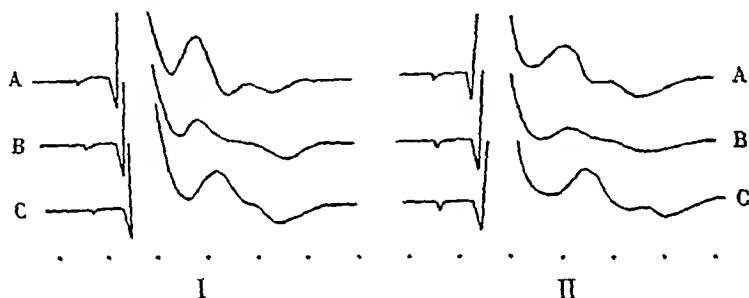


Fig. 2. Decerebrated cat. Action potentials set up by maximal stimuli 5 per sec. I: A, before; B, 70 sec. after 25 μ g. adrenaline, showing reduction of δ spike; C, recovery of δ spike. II: A, before; B, during occlusion of aorta for 65 sec.; C, 1 min. after release of aortic clamp. Time: 1 d.v. = 2 msec.; 1.0 mV. = 1 cm.

varied in different cats; in some cats the effect could be readily demonstrated with 5 μ g. adrenaline only, in others 25 μ g. would produce the effect. However, within the same animal, the same dose of adrenaline injected intravenously or into the artery of the opposite leg produced only about half the effect seen with adrenaline injected into the same leg; although the rise in blood pressure and consequently the improvement in the general circulation was the same or greater. The increase of the $\alpha\beta$ spike began after 1–1½ min.; it reached its maximum usually at 2 min., i.e. when the effect on the blood pressure was passing off, and disappeared after 3–5 min. This effect of adrenaline on $\alpha\beta$ spikes could not be reproduced by clamping the aorta for 1 min., which had no effect on the threshold at frequencies up to 200 per sec. The adrenaline effect could, however, be prolonged when the aorta was clamped 10 sec. after the injection of adrenaline. In one experiment the increase of the $\alpha\beta$ response which previously had disappeared in 3½ min., now persisted until the

aortic clamp was removed after 4 min., when it disappeared $\frac{1}{2}$ min.

The lowering in threshold by adrenaline was also evident in δ with submaximal stimuli at rates up to 40 per sec. In one experiment with maximal stimuli at 1 per sec. $\alpha\beta$ and δ spikes were quite unaffected by 25 μ g. adrenaline; at a rate of 20 per sec. the δ spikes were absent 1 min. after the injection of 25 μ g. adrenaline and had returned to normal

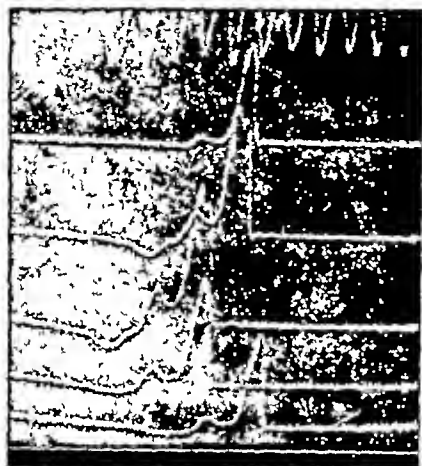


Fig. 3.

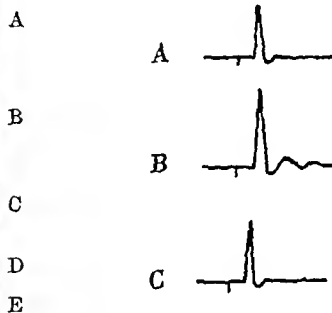


Fig. 4.

Fig. 3. Spinal cat, eviscerated, adrenalectomized. Action potentials set up by 25% of maximal at 1 per sec. A, before; B, 40 sec. after 5 μ g. adrenaline; C, 1 $\frac{1}{2}$ min.; D, after 2 $\frac{1}{2}$ min.; E, after 3 $\frac{1}{2}$ min. Time: 1 d.v. = 2 msec.; 0.4 mV. = Read from right to left.

Fig. 4. Same experiment as Fig. 1. Action potentials set up by submaximal stimuli 1 per sec. A, before; B, 3 min. after 20 μ g. adrenaline; C, 5 min. after. Time: 1 d.v. = 10 msec.; 0.7 mV. = 1 cm.

after 3 min. (Fig. 1); the $\alpha\beta$ spikes remained unchanged. Immediately afterwards with stimuli 20% of maximal at 36 per sec. both $\alpha\beta$ and δ spikes increased in amplitude after the injection of 20 μ g. adrenaline, reaching their greatest height after 3 min. and falling again in 5 min. (Fig. 4).

Other methods were tried of raising the blood pressure. Intravenous injection of 20 c.c. normal saline caused a rise of blood pressure to the same extent as did 20 μ g. adrenaline, but had no effect on the height of the response of the nerve to submaximal stimuli, though it increased muscle tension. No effect was observed with ephedrine in doses of 1

2 mg. In one experiment 0.5 unit pituitary (posterior lobe) extract caused an increase of 80 % in height of nerve response which lasted for over 30 min., in fact as long as the rise in blood pressure and the increase in muscle tension persisted. In another experiment 7.5 mg. ergotoxine abolished the increase in α spike height with submaximal stimuli produced by adrenaline, and reversed the effect on δ spikes due to maximal stimuli.

While we have never failed to obtain with adrenaline an increase in the height of response of $\alpha\beta$ fibres to submaximal stimuli with the nerve in the best possible condition, the effect was much greater when there was evidence of fatigue. In nerves in good condition the effect varied from 30 to 115 %; but at the end of an experiment on a spinal animal, when the blood pressure had fallen to 30 mm. Hg, increases of response from 10 μ V. to 1 mV. have been seen. This was present with stimuli at 1 per sec. In early experiments, where the nerve had been incompletely guarded from fatigue, increases of 300 % in height of response were frequently observed. A small increase in the power of β fibres to respond to stimuli at 250 per sec. was seen in a fatigued nerve after adrenaline.

When muscle tension and nerve action potentials were recorded, a striking absence of parallelism between the effect of adrenaline on muscle and nerve was observed. For instance, when no effect on the response in nerve could be seen with maximal single shocks at 1 per sec., 100 % increase of muscle tension could be observed when the muscle was fatigued. On the other hand, with maximal interrupted tetani of high frequency, 25 μ g. adrenaline in the same experiment completely abolished the muscle tension for 1 min. after the injection, again without any change in the $\alpha\beta$ spike potential. When with a maximal tetanus of 20 per sec. complete abolition of the δ spikes occurred within 1 min. after the injection of adrenaline, the muscle tension rose at the same time by 160 %. And when with submaximal stimulation there was an increase in muscle tension it always began earlier and outlasted the increase in the nerve response; sometimes a transient decrease of muscle tension was observed while the nerve action potential was growing. A further investigation of the effect of adrenaline on the nerve muscle junction is called for.

DISCUSSION

It appeared from the experiments of Bülbring & Burn [1939] that adrenaline had probably an effect on excitation of the motor roots in the isolated perfused hind limb of the dog. We have now obtained an increase in the spike height of the normal sciatic nerve in cats, which must be

chiefly due to a change of threshold. The effect of fluctuation in the inter-electrode resistance has been minimized by the use of a resistance of $50,000\Omega$ in series with the stimulating circuit and the electrodes. An effect of the magnitude of that described could not be due to any decrease in inter-electrode tissue fluid possible within physiological conditions.

The possibility remains that adrenaline produced an increase in the K/Ca ratio in the nerve which was responsible for the shift in threshold. We have at no time seen repetitive discharge at the height of the adrenaline effect. Its onset is also very rapid compared with the effect of changes in pH and calcium-ion concentration described by Lehmann [1937 *a*, *b*].

The nerve was isolated with great care, with minimal disturbance of its blood supply, and stimulation was avoided as far as possible before making observations. Therefore, we believe that, although the effect described may reach a much greater height in fatigued nerve, adrenaline can produce a lowering of threshold in healthy nerve. There was some evidence that adrenaline increased the rate of recovery of excitability of nerve, possibly by accelerating processes concerned with supernormality [Graham & Lorente de N6, 1938]. This could be more easily studied if the phenomenon were reproducible *in vitro*.

In decerebrate preparations with intact suprarenals, fluctuations of threshold were frequently observed which could be attributed to the discharge of adrenaline from the suprarenal glands. It is possible that this has been one of the difficulties in the study of the excitability of mammalian nerves *in situ*.

The depression of δ spikes can be reproduced by occluding the blood supply, and it is synchronous with the greatest vasoconstriction in the blood vessels of the perfused nerve (Bülbring, unpublished observations). It is therefore to be attributed to anoxaemia, to which δ fibres are almost as susceptible as B fibres [Grundfest, 1939].

SUMMARY

1. The effect of adrenaline has been studied on the excitability of the cat's sciatic nerve *in situ*.

2. In healthy nerve intra-arterial injection of $5\text{--}25\mu\text{g}$. adrenaline increases the height of the action potential produced by submaximal stimuli. This effect is due to a lowering of threshold; it lags behind and outlasts the vascular action of adrenaline.

3. The effect of adrenaline is much larger when the nerve shows fatigue.

4. The same doses of adrenaline reduce or abolish the δ spike in the action potential produced by maximal stimuli; this effect is attributed to the reduction of blood flow caused by adrenaline.

The authors wish to thank the Christopher Welch Fund Trustees for defraying the cost of photographic materials used in this work.

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THE UTILIZATION OF FAT BY THE AGLYCAEMIC MAMMALIAN HEART

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THE investigation of fat utilization by an organ which has so definite a storage of fat and carbohydrate as the mammalian heart demands the withdrawal of carbohydrate both from the heart and the blood perfusing it. The first part of this work was confined to determining the relation between heart glycogen and blood sugar in order to assess the degree of aglycaemia produced in the isolated heart-lung preparation in the rat, the second part to the investigation of fat utilization by the heart.

PART I. THE AGLYCAEMIC HEART-LUNG PREPARATION

Many investigators have shown that, with a marked fall in blood sugar, there is a decrease in cardiac glycogen [Cruickshank & Startup, 1933; Bogue, Evans, Grande & Hsu, 1935; Cruickshank & McClure, 1936; Chang, 1937, etc.]. Visscher & Mulder [1930] and Fletcher & Waters [1938] are amongst those who do not support this contention. Differences in opinion on this point have arisen because primarily conclusions have been drawn with regard to the retention or loss of heart glycogen under varying degrees of hypoglycaemia. It has been shown, on the one hand, that a slight hypoglycaemia effects little if any change in heart glycogen, while on the other a marked hypoglycaemia, i.e. a blood sugar of 10 mg./100 c.c. of blood, may cause a loss of heart glycogen amounting to 75%. In the course of the work to determine whether or not a definite relation existed between the amount of cardiac glycogen and the final percentage of blood sugar, the specific action of adrenaline on cardiac glycogen was also noted.

METHODS

The work was carried out on male Lister strain rats, between 250 and 350 g. weight. The animals were kept on a fixed and ample diet; all, bleeders and experimental rats alike, were starved 24 hr. previous to the experiment. Since the animals were actively growing there could be no question of their freedom from old-age fat. The hearts weighed from 0.50 to 1.00 g., and made excellent isolated heart-lung preparations.

In making the isolated heart-lung preparation, the only divergence from the usual method in the dog was that the venous blood was brought into the right auricle by way of the inferior vena cava; the azygos vein was clamped at its junction with the left superior jugular. Jorpes's heparine was used as an anticoagulant. Blood was not put into the incubator until the commencement of the experiment. The total amount of blood in circulation varied from 12 to 14 c.c.; in relation to the size of the heart (0.5-1 g.) it should be noted that this is not a small volume of blood. As the venous reservoir was a glass tube 5 in. \times $\frac{1}{4}$ in. and holding 4 c.c. of blood, an accurate measurement of venous pressure and blood loss could be made. The remainder of the blood is contained in the resistance chamber, in the glass and rubber tubing and in the heart and lungs. Since the average weights of the heart and both lungs are 0.7 and 0.4 g. respectively, the amount of blood within them can be taken as approximately 0.5 c.c. Artificial respiration was carried out with a small Ideal type of pump made for the purpose by Messrs Palmer & Co.: the stroke varied between 3 and 4 c.c. Blood sugar was estimated by the Hagedorn-Jensen method using the micro-technique of Somogyi [1931]. For glycogen determinations the modifications for micro-estimations of the original Pfüger method, put forward by Cori [1932] and Good, Cramer & Somogyi [1933] were employed. The heart was rapidly removed at the end of the experiment, trimmed of aorta and pericardium, ventricles opened, mopped dry of blood and immediately immersed in liquid air. When hard the muscle was weighed, returned to the liquid air and ground to a fine powder. A dry-weight determination was always made. Lactic acid in blood was estimated by the method of Lehnartz [1929].

RESULTS

The glycogen content. Determinations of normal heart glycogen were made on ten controls. The dry weight of the heart varied from 20.40 to 24.60% of the wet weight with an average of 23.9. The wet weight figures for glycogen content varied from 460 to 567 mg./100 g. of tissue

with an average of 520 mg./100 g., while the dry-weight percentages were very constant, varying from 1.98 to 2.40 g./100 g. dry muscle, with an average of 2.22 g./100 g. The average blood sugar content was 94.3 mg./100 c.c.

The relation of blood sugar to glycogen content (dry). The figures in Table I show the relation: percentage blood sugar to percentage cardiac glycogen (dry) at the end of stated experimental periods. That a definite logarithmic relation exists is evident from Fig. 1, where it is shown that the final glycogen content varied as the logarithm of the final blood

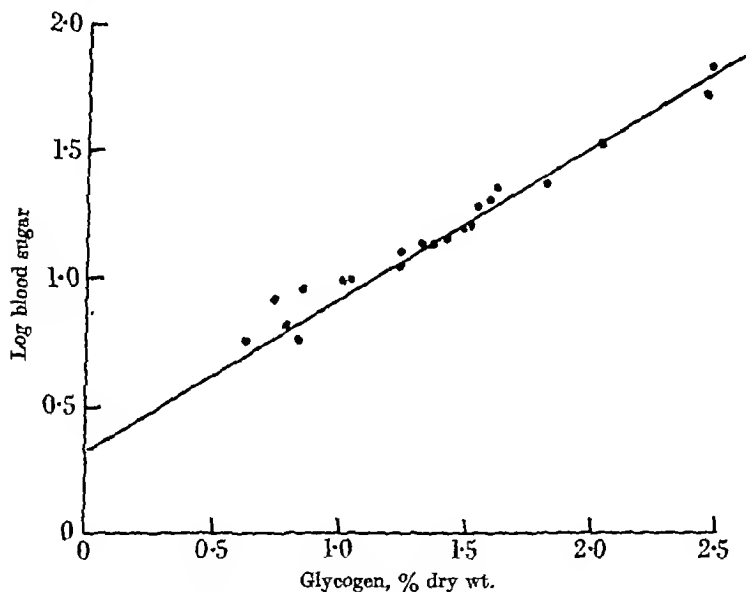


Fig. 1. Showing the logarithmic relation between blood sugar and cardiac glycogen.

sugar. In individual experiments the rate of these hearts ranged from 240 to 360 beats per min.; the venous pressure from 2.5 to 6.5 cm. of blood, the volume of blood in circulation from 12 to 14 c.c.

The results given in Table I are arranged according to a decreasing percentage of final blood sugar. A condition of hypoglycaemia in which the blood sugar is 53.81 mg./100 c.c. blood is not associated with a loss of heart glycogen. It is seen from Table I that only after 1 hr. does an appreciable hypoglycaemia (33.98 mg./100 c.c.) supervene, and that at least $1\frac{1}{2}$ hr. perfusion are required under the conditions obtaining, to secure so marked a hypoglycaemia, viz. 25.12 mg./100 c.c. blood, that a loss of one-third to one-half of the heart glycogen will take place.

TABLE I. Table showing the relation of cardiac glycogen (% dry weight) to blood sugar (mg./100 c.c.) at the end of the experiment

Exp.	Blood sugar mg./100 c.c.	Log blood sugar conc.	Glycogen % dry wt.	Duration of experiment hr.
21	68.7	1.837	2.21	$\frac{1}{2}$
14	53.8	1.731	2.19	$\frac{1}{2}$
12	33.9	1.531	1.90	1
7	25.0	1.398	1.80	$1\frac{1}{2}$
20	22.6	1.354	1.61	$1\frac{1}{2}$
1	21.2	1.326	1.58	2
16	19.8	1.297	1.45	$1\frac{1}{2}$
8	16.9	1.230	1.40	2
10	16.9	1.230	1.34	2
2	14.3	1.156	1.40	2
13	14.1	1.151	1.23	$1\frac{1}{2}$
15	14.1	1.151	1.20	$1\frac{1}{2}$
17	12.2	1.088	1.21	$1\frac{1}{2}$
4	10.4	1.020	0.95	2
11	10.0	1.001	0.94	2
3	9.3	0.968	0.84	3
5	9.2	0.963	1.12	2
18	8.4	0.928	0.74	2
6	6.5	0.816	0.71	$2\frac{1}{2}$
9	5.6	0.752	0.75	2
19	5.6	0.752	0.63	2

Ten controls: Heart glycogen = 2.22 g./100 g. dry wt. (1.98-2.40).

Blood sugar = 94.33 mg./100 c.c. blood.

Only after 2 hr. perfusion, when the blood sugar is reduced to less than 10 mg./100 c.c. blood, can the preparation be regarded as virtually aglycaemic, that is, a condition in which the blood sugar is less than 10 mg./100 c.c. blood and the heart glycogen less than 1.0 g./100 g. of dry heart muscle.

The effect of adrenaline on the glycogen: blood sugar relation. The results in Table II bear no resemblance to the relationship shown in Table I. They would indicate that the continuous addition of adrenaline,

TABLE II. Table showing effect of adrenaline on the relation of cardiac glycogen to blood sugar and on sugar and glycogen utilization

Duration of experiment 1 hr.

Exp.	Blood sugar mg./100 c.c.	Glycogen %	Utilization mg./100 g./hr.		Adrenaline 1 : 1000 c.c.
			Glucose	Glycogen	
7	29.0	0.62	549	450	0.6
5*	42.4	0.97	637	385	0.4
2	28.3	0.68	741	397	0.4
8*	49.4	0.78	775	328	0.7
6	31.1	1.12	854	335	0.8
3	36.8	0.82	1056	410	0.5
4	39.6	0.89	1255	405	0.5
1	30.0	0.92	1345	349	0.8

* Duration 45 min.

in the amounts shown in the table, has a specific action upon cardiac glycogen, since the loss in glycogen is far greater than that which would normally be produced by a similar final concentration of blood sugar. They further show that an amount of adrenaline which would produce an aglycaemic heart within 1 hr. would not reduce the blood to the aglycaemic level in that time.

The utilization of glucose, lactic acid and glycogen. A perusal of the figures in Table III will show how varied can be the utilization of

TABLE III. Showing effect of changes in heart rate, blood pressure, venous pressure and duration of the experiment on the utilization of blood sugar and cardiac glycogen

Exp.	Total glucose used mg.	Glucose used mg./100 g. heart muscle per hr.	Glycogen used mg./100 g. heart muscle per hr.	A.B.P. mm. Hg	Heart rate b.p.m.	Drops/min. 1 drop= 0.075 c.c.	Venous pressure cm. blood	Time hr.
10	2.8	141	132	90	280	60-40	2.5	2
6	3.2	150	152	100	224	70-40	2.5	2½
8	2.5	165	126	120	300	85-50	3	2
7	1.8	181	102	90	240	80-50	3	1½
9	3.8	189	184	120	255	60-40	3	2
11	3.9	194	171	120	320	60-50	3	2
4	4.9	202	185	80	240	90-55	3	2
3	4.5	231	120	100	220	80-60	3	3
5	4.3	236	160	100	240	80-60	3	2
1	3.9	280	116	75	250	108-65	3	2
13	5.6	320	175	120	240	90-70	3	2
2	4.8	370	104	80	200	80-50	4	2
18	3.8	377	193	120	360	140-130	6	2
20	3.4	435	180	120	300	100-90	5	1½
14	1.6	454	0	80	240	85-85	5	½
12	5.1	488	90	120	250	90-70	5	1
19	5.2	469	201	135	360	130-130	8.5	2
17	5.9	470	136	120	320	110-100	5.5	1½
15	5.1	485	168	100	260	120-90	5.5	1½
16	5.7	543	138	120	300	120-100	6	1½

carbohydrate by the heart and lungs of the rat, and how the balance between blood sugar and glycogen is dependent upon various factors, for example, blood pressure, venous inflow, venous pressure, heart rate, and the duration of the experiment. The data in this table are arranged in the order of a rising glucose utilization. The output of the heart can be well maintained by keeping the venous pressure constant. This is easily done, because usually there is no loss of blood during the first hour of the experiment. Later, slight oedema of the lungs gives rise to a slow fall in venous pressure which, if adjusted by raising the reservoir, restores the heart output at once to the original figure. The total loss of

blood in a 2 hr. experiment may be 0.5–2 c.c., depending chiefly upon the arterial blood pressure, which, if too high, is responsible for a more marked lung oedema. One drop of blood with a constant size of outlet was equal to 0.075 c.c. From Fig. 1, on extrapolation, it might be inferred that, when the glycogen is exhausted, the blood sugar content is not more than 2.4 mg./100 c.c. This represents a titration figure of 0.02 c.c. of 0.005 *N* sodium thiosulphate, which is within the experimental error of the method for blood sugar estimation.

As a result of these experiments it became evident that the best cardiac action could be obtained and maintained for at least 2 hr. if the arterial pressure, measured just beyond the aortic cannula, was kept at about 100 mm. Hg and the venous pressure at 5 cm. of blood. Under these conditions the heart rate was generally about 360 beats per min. At the end of 2 hr. the heart is always actively beating, and shows no marked variation in rate if the venous inflow pressure is maintained at not less than 5 cm. of blood. When a heart has lost 70 % of its glycogen and is actively beating with a blood sugar content of approximately 10 mg./100 c.c. of blood, it is apparent that carbohydrate as a source of energy has virtually disappeared from the blood.

An examination of the results in Table III leads to some surprise at the great variation in sugar utilization. In assessing the comparative value of these figures, due attention must be paid to the weight of the heart, the level of blood sugar at the beginning and end of the experiment, the arterial and venous pressures and the rate of the heart. The apparent discrepancy between, for example, exps. 9 and 18, will be clarified by noting the experimental data:

	Exp. 9	Exp. 18
Wt. of heart	1.00 g.	0.50 g.
Dry weight	22.95 %	20.85 %
Blood sugar	71.00/5.66 mg./100 c.c.	89.20/8.49 mg./100 c.c.
Glycogen	71.00/32.66 mg./100 c.c.	89.20/35.40 mg./100 c.c.
A.B.P.	120 mm. Hg	120 mm. Hg
Venous pressure	3 cm.	6 cm.
Heart rate	255 b.p.m.	360 b.p.m.
Volume of blood	14 c.c.	14 c.c.
Blood sugar used	27.00 × 0.14 3.78 mg./2 hr. <u>= 189.00 mg./100 g./hr.</u>	26.91 × 0.14 3.77 mg./2 hr. <u>= 377.00 mg./100 g./hr.</u>
Glycogen content of heart:		
Beginning	5.40 mg.	2.70 mg. (cal.)
End	1.71 mg.	0.77 mg.
Glycogen used	3.69 mg. <u>= 184.5 mg./100 g./hr.</u>	1.93 mg. <u>= 193 mg./100 g./hr.</u>

Final total glycogen in heart in mg. = glycogen % × dry wt. % × wt. of heart/10
 Glycogen at the beginning in mg. = av. % (2.22) × av. dry wt. (23.9)/10
 = 5.4 × wt. of heart

The considerable differences in sugar utilization are matched by similar differences in lactic acid utilization. The amounts of lactic acid used are, with a few exceptions, in the reverse order to those of glucose (Table IV and Fig. 2) [see Evans, Grande & Hsu, 1935]. As noted with sugar utilization, several factors again play a part in determining how

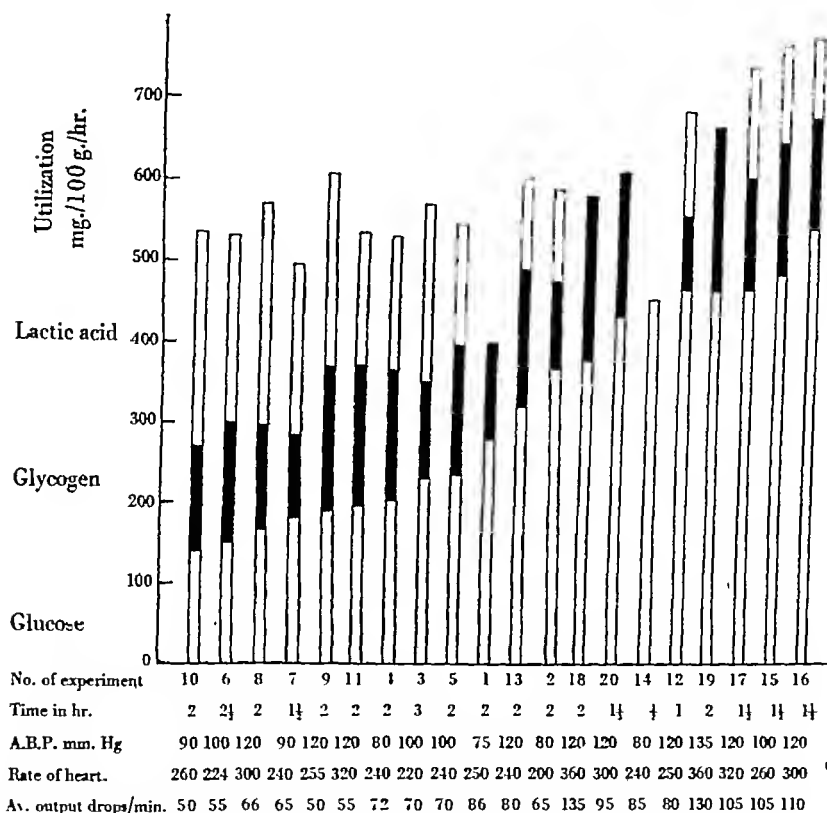


Fig. 2. Showing the relation between glucose, glycogen and lactic acid utilization under stated experimental conditions.

much lactic acid may be used in the course of an experiment. The two main factors are the total amount of blood sugar present at the beginning of the experiment and the rate at which the sugar is used. The utilization is determined by the work done by the heart and the duration of the experiment. Table IV shows this inverse ratio, the amount of glucose used, ranging from 141 to 543 mg./100 g. of heart muscle/hr., lactic acid varying from 276 to 100 mg./100 g. of heart muscle/hr. It also shows

TABLE IV. A comparison of glucose, glycogen and lactic acid utilization, mg./100 g./hr.

Exp.	Duration hr.	Glucose	Lactic acid	Glycogen	Total
10	2	141	265	132	538
6	2½	150	229	152	531
8	2	165	276	126	567
7	1½	181	212	102	495
9	2	189	236	184	609
11	2	194	163	174	536
4	2	202	160	165	527
3	3	231	220	120	571
5	2	236	149	160	545
13	1½	320	111	175	606
2	2	370	115	104	589
12	1	468	134	90	692
17	1½	470	140	136	746
15	1½	485	121	168	774
16	1½	543	100	138	781
Av.		289.6	175.7	141.8	607.1

that the amount of lactic acid used depends, in these cases, on the duration of the experiment, since the longer the experiment the smaller, generally, will be the amount of sugar used per 100 g. of heart muscle per hr., and the greater will be the degree of glycolysis, with a consequent greater possibility of lactic acid utilization. A point of interest which may be mentioned, in that it confirms a similar statement by Evans, Hsu & Kosaka [1934], is that in the few experiments in which lactic acid was estimated at the end of 1 hr., there was always an increase above the amount produced by glycolysis as determined from the incubated blood sample. This is indicative of the production of lactic acid by the lungs, and was particularly noticeable in the experiments where the glycogen was rapidly reduced by the addition of adrenaline. Taking the average of the figures in Table IV, we find that glucose, lactic acid and glycogen show utilizations of 289.6, 175.7, 141.8 mg./100 g./hr. respectively. Evans *et al.* [1935] discussed the combined utilizations of glucose and lactic acid in the heart of the dog and, finding great variations in each, and a reciprocal relation of the one to the other, they considered them as a common pabulum since they have much that is common in their metabolic history. Similarly, one can include glycogen in this category of substances of common metabolic activity, and doing so we find the following range of utilization of each:

Utilization mg./100 g./hr.	Glucose	Lactic acid	Glycogen	Total
Average	289.6	175.7	141.8	607.1
Range	141-543	100-276	90-184	495-781
Ratio max. : min.	3.85	2.76	2.04	1.57

The sum of glucose, lactic acid and glycogen shows a smaller range of fluctuation than any of the three components. This greater range of glucose utilization compared with that of lactic acid and glycogen may be interpreted as indicating a tendency of the heart and lungs to use glucose while it is available, and for the heart to make good its energy requirements from, first lactic acid and then glycogen, when faced with a rapidly diminishing blood sugar concentration.

The restitution of cardiac glycogen. It has been shown that glycogen can be spared by the addition of either glucose or lactic acid to the blood. Knowing from the blood sugar concentration the extent to which the glycogen of the heart may be reduced, one can demonstrate the restoration of the lost glycogen by the addition of glucose to the perfusing blood. As the following figures show, a glycogen loss of 76 % in 2 hr. can be restored to 83 % of its original value by raising and maintaining the blood sugar above the normal content. In the experiment noted below, the blood pressure was kept at 135 mm. Hg, and the venous pressure at 6 cm. of blood for 2 hr. The rate of the heart was 360 b.p.m. during this period. At the end of 2 hr. the blood sugar had fallen to 4.60 mg./100 c.c. of blood, which corresponds to a glycogen content of 0.58 g./100 g. dry tissue. After the addition of glucose the blood pressure was reduced to 80 mm. Hg, the venous pressure being kept at 6 cm. of blood. An hour later a vigorous heart was removed for the estimation of its glycogen, which was 2.06 g./100 g. dry weight.

Weight of heart 0.6 g. Dry wt. 21.86 %		
Time hr.	Blood sugar mg./100 c.c.	Glycogen total in mg.
0.00	93.4	3.24
2.00	4.6	0.76*
Glucose added		
2.05	207.0	—
3.00	111.6	2.70

Glycogen restitution = 83.3 %.

* Calculated from data in Fig. 1.

PART II. THE UTILIZATION OF FAT BY THE HEART OF THE RAT

Evidence for the oxidation of fatty acids by heart muscle has, up to date, been inconclusive. That fat can be utilized to supply energy for skeletal muscle has been indicated by Benedict & Cathcart [1913] and by Krogh & Lindhard [1920]. Lovatt Evans [1914] has shown that when animals have been fed on a diet consisting largely of butter, low respiratory quotients are obtained in experiments upon the isolated heart.

That a change from carbohydrate to fat metabolism is a possibility in muscle activity has been suggested by the work of Meyerhof & Boyland [1931] on the frog's sartorius, poisoned with iodo-acetic acid, by Gemmill [1934] in his work on energy supply in the human subject on low carbohydrate high fat diets, and by Cruickshank & McClure [1936] who, using the aglycaemic isolated heart-lung preparation on the dog, state that since the heart, deprived of glucose, fails to make use of circulating amino-acids, shows a falling CO_2 output, and a respiratory quotient of 0.7, the possibility of a direct oxidation of fat must be admitted. Visscher [1938], after a long series of experiments, tends also to the conclusion that fat can be burned by the mammalian heart. Fletcher & Waters [1938] state that none of the blood fat is utilized by the heart. If this were true, fat as a source of cardiac energy would be in a different category from cardiac glycogen, and the question of the origin of cardiac fat would be immediately raised. Attempts to relate oxygen consumption to fat utilization have not resulted in any conclusive evidence for direct oxidation of fat.

In the present investigation changes in blood sugar and cardiac glycogen have been related to changes in the blood and cardiac fatty acids in the aglycaemic heart-lung preparation in order to determine to what extent the heart may be made to utilize fatty acids.

METHODS

Fatty acids in heart muscle and blood were estimated in addition to the substances dealt with in Part I. Owing to the small quantities of heart muscle and blood available, a gravimetric method was inapplicable. Although estimation by acidimetry would have been possible, it was not considered satisfactory because, among other reasons, it takes no account of the possibility of alteration of the molecular weight of the fatty acids by oxidation. It was decided, therefore, to estimate the fatty acids by oxidation with chromic acid. Phospholipins were separated from neutral fat and cholesterol by precipitation with acetone, and the fatty acids were estimated separately [Bloor, 1917, 1928, 1929]. Minor modifications which were introduced are described below. All general precautions recommended by Bloor were carefully observed, particularly with regard to solvents. The conditions of oxidation were carefully checked by using pure palmitic acid. It was found that 25 min. at $124-126^\circ$ were required to ensure complete oxidation. The final evaporation of all solvents was carried out under a current of nitrogen.

Heart extraction. The method is a modification of that described for muscle by Bloor & Snider [1934]. About 0.5 g. of heart muscle is ground under liquid air, then reground with ignited sand, 1–2 c.c. of water being added. The mass is then transferred to a 100 c.c. conical flask, the mortar washed with 3 : 1 alcohol-ether mixture and the muscle extracted with 25–30 c.c. of the same mixture on the boiling water bath for 3 min. The extract is poured through fat-free filter paper (Whatman No. 43) into a 100 c.c. graduated flask. The extraction is repeated twice and finally once with ether alone and the volume made up to 100 c.c. with alcohol.

Cholesterol estimation. The method is that of Bloor [1917, 1928]. The standard is 0.25 mg. cholesterol in chloroform; this is kept at 22° for 15 min. and at the same light conditions as will prevail at the time of estimation.

Phospholipin and neutral fat fatty acids. Thirty-five c.c. of the alcohol-ether extract are evaporated to dryness in a 100 c.c. beaker. The residue is dissolved by warming it gently with three small portions of petroleum ether; the extract is decanted into a 15 c.c. centrifuge tube graduated at 2 and 10 c.c.; the final volume is 10 c.c. After concentrating the extract to 2 c.c. by immersing in a little hot water, 7 c.c. of dry redistilled acetone and three drops of 4% $MgCl_2$ in alcohol are added. Separation of phospholipin and neutral fat is obtained by centrifuging for 2 min. at 1500 r.p.m. The supernatant acetone (neutral fat) is decanted into a 100 c.c. conical flask, the precipitate (phospholipin) is twice washed with a 2 c.c. acetone, centrifuged and the acetone decanted. The acetone solutions are taken to dryness, and about 15 c.c. alcohol-ether (3 : 1) and 0.1 ml. of saturated NaOH are added. The mixture is saponified for 1½ hr. The precipitate is dissolved in 5 c.c. warm alcohol-ether and transferred to a 100 c.c. flask. This is done twice, making sure to transfer all solid particles. Saponification is carried out with 0.1 ml. saturated NaOH for 1½ hr. After saponification the oxidation, with dichromate, was carried out as described by Bloor [1928, 1929].

In the calculation the c.c. dichromate corresponding to cholesterol are subtracted. The resulting titre is divided by 3.6 (3.6 c.c. 0.1 *N* dichromate = 1 mg. fatty acid).

Estimation of total fatty acids in blood

Blood. Into 30 c.c. of alcohol in a 50 c.c. volumetric flask, 3 c.c. of blood are run in a slow stream with continuous rotation of the flask. The precipitate must be finely flocculent. The flask is immersed in boiling water for 3 min., rotating it continuously to prevent bumping. After the contents have begun to boil, 10 c.c. of ether are added and the mixture boiled again for a few seconds. It is then cooled in running water to room temperature, made up to 50 c.c. with alcohol-ether, and filtered through folded fat-free filter paper into a dry Erlenmeyer flask, the filtrate being pressed out with a glass rod. Saponification in duplicate is carried out on 20 c.c. of the filtrate. The petroleum ether extract is transferred to a 25 c.c. volumetric flask. Ten c.c. are used for oxidation; the remaining 15 c.c. in the duplicates are combined and 25 c.c. are taken for cholesterol estimation.

The blank consists of 25 c.c. alcohol-ether, it is saponified, transferred to a 25 c.c. volumetric flask and two portions of 10 c.c. each taken for oxidation and subsequent titration.

Unfortunately the cholesterol estimation in heart muscle by the Liebermann reaction is inaccurate, since the colours of the standard and unknown often fail to match. It was, therefore, impossible to obtain an accurate value for the neutral fat fatty acids, i.e. oxidation value of the acetone soluble fraction minus that of the cholesterol. For this reason one has to compare the quantities of *N* dichromate required for the oxidation of neutral fat fatty acids and cholesterol per 100 g. dry tissue. In Tables V and VI this value is called the "titre". It should be noted in Table V that the figures for neutral fat fatty acid are obtained by subtracting the estimated figures for cholesterol from those calculated

from the neutral fat fatty acid plus cholesterol titration. The ideal method would be the isolation of cholesterol by the digitonin method followed by oxidation. This would make possible a direct estimation of cholesterol, and, by difference, would give a reliable figure for the neutral fat fatty acids. The small quantity of material available made this procedure impracticable, at least, for a preliminary investigation.

RESULTS

Controls: phospholipin and total fatty acid in heart muscle

In Table V are given the control estimations in eight hearts. The last column gives the titre per 100 g. of dry heart muscle of the neutral fat fatty acid plus cholesterol. This has been done because in perfusion

TABLE V. Control estimations of cholesterol, phospholipin, total fatty acid, and neutral fat fatty acid plus cholesterol in the heart of the rat. Dry weight

Exp.	Dry weight %	Cholesterol g./100 g.	Phospholipin fatty acid g./100 g.	Neutral fat fatty acid g./100 g.	Neutral fat fatty acid + cholesterol titre c.c. N dichro- mate/100 g.
1	23.76	0.76	7.9	1.23	759
2	24.20	0.77	7.1	1.22	740
3	24.20	0.89	8.4	1.50	859
4	24.14	0.83	5.9	1.47	874
5	24.07	1.15	7.5	0.80	735
6	23.37	0.89	8.6	1.91	1034
7	23.35	—	7.3	—	847
8	23.60	—	7.7	—	843
Av.		0.89	7.6	1.36	839

experiments the cholesterol was not separately estimated, as it was found that the method employed was not sufficiently accurate for its estimation. In subsequent experiments the combined "titre" is also given. For reasons already stated this gives more accurate comparative titration figures. The average figures for phospholipin fatty acid and neutral fat fatty acid are 7.6 and 1.36 %, while for the combined neutral fat fatty acid and cholesterol the titre, in c.c./100 g. of dry heart muscle, ranges from 735 to 1034, with an average of 839.

Perfusion experiments (Table VI). In these experiments the constancy of the phospholipin fatty acids is noteworthy; the figures range from 7.0 to 8.3 % with an average of 7.7 %, which is in agreement with the figure for the controls. The cholesterol was found not to vary over a range any greater than that shown in the controls. To indicate the loss in heart muscle fatty acids, the average heart neutral fat fatty acid of the controls, viz. 839 c.c./100 g. of muscle is contrasted with the finding for each experiment, the percentage loss being given in the subsequent column. These values must, of course, be viewed in the light of what has

been stated concerning the estimation of cholesterol. The percentage loss (column 9), while not great, is quite definite, and its relation to the loss of blood sugar and cardiac glycogen is shown in the table (columns 3 and 6). That the hearts in these experiments are almost aglycæmic is shown by giving in mg. the total amount of sugar in the blood and glycogen in the heart at the end of the experimental period (columns 2 and 5, Table VI). In these experiments the blood pressure varied between 100 and 120 mm. Hg, the venous pressure between 4 and 5 cm. of blood. The utilization figures for blood sugar and heart glycogen are in accordance with those mentioned in the first part of the paper; the average figures for the percentage loss of blood sugar, heart glycogen and heart fatty acids are shown and would justify the general conclusion that a loss of 80 % of blood sugar results in a loss of 45 % of heart glycogen and 25 % of the fat of the heart. Since cardiac glycogen and fat may be regarded in the same category of energy sources for heart muscle, the next step was to investigate the effect upon blood fatty acids when a heart was made to increase its utilization of blood sugar and glycogen by raising the venous pressure from 4 to 7 cm. of blood.

Utilization of blood fatty acids. In Table VII are set out the figures for utilization of blood sugar, heart glycogen and blood fatty acids, with their respective percentage loss at the end of the experimental period. From these results it is clear that an increased blood sugar utilization is associated with an increased venous pressure. In the 2 hr. experiments where venous pressure was raised from 4 to 7 cm. of blood, the utilization of blood fatty acids rose from 203 to 957 mg./100 g./hr., the percentage loss from 14.8 to 47.1. It is again demonstrated in these experiments that fat utilization follows an almost complete depletion of blood sugar, and that the extent to which fatty acids will be used by the heart depends upon the degree to which cardiac glycogen has been reduced. For the 2 hr. experiments the average percentage losses for blood sugar, heart glycogen and blood fatty acids were 83.9, 58.2 and 28.5 respectively. In general terms one may state that the *maximum* losses of blood sugar, heart glycogen and blood fatty acids in this period of time would be 95, 75 and 50 % respectively (exp. 7, Table VII). The remaining experiments show that a loss of about 50 % of the glycogen of the heart must take place before any utilization of blood fat by the heart can occur. Where a 40 and 47.1 % utilization of blood fatty acids occurred, as in exps. 6 and 7, the total amounts of blood sugar remaining were 1.2 and 0.7 mg. and the final total content of cardiac glycogen 0.74 and 0.84 mg. respectively. The results of these experiments are interpreted as indicating

Exp.	Blood sugar			Heart glycogen			Heart neutral fat fatty acids + cholesterol		Heart phospholipin fatty acid g./100 g. dry wt.
	Final total mg.	% loss	Utilization mg./100 g./hr.	Final total mg.	% loss	Utilization mg./100 g./hr.	Titre c.c./100 g./dry wt.	% loss	
1	2.3	74.5	261	2.8	41.7	123	565	32.6	7.0
1	2.0	86.3	208	2.4	46.6	130	633	24.6	7.6
2	1.3	76.7	251	3.1	48.3	130	620	26.2	8.2
3	1.2	87.1	314	2.2	63.3	177	645	23.2	8.3
4	2.5	78.0	543	2.6	33.3	138	614	26.8	7.4
Av.	1.8	79.3	327.4	2.6	46.6	141.6	615	26.7	7.7

Duration of exps. = 2 hr., except exp. 5 = 1½ hr.

Col. 9: % loss = $\frac{(c - \text{titre}) \times 100}{c}$.

c = control average of neutral fat fatty acids plus cholesterol in seven experiments = 839.

TABLE VII. Showing utilization and percentage loss of blood sugar, cardio glycogen and blood fatty acids, arranged in the order of an increasing blood sugar utilization

Exp.	Blood sugar			Heart glycogen			Blood total fatty acids			Venous pressure cm.	Heart rate b.p.m.	Time hr.	
	Utilization		% loss	Utilization		% loss	mg./100 c.c.		Utilization mg./100 g./hr.				% loss
	mg./100 g./hr.	% loss		mg./100 g./hr.	% loss		Beginning	End					
3	251	76.7	130	48.3	193	167	203	14.8	4.0	260	2		
1	261	74.5	122	41.7	181	153	181	15.4	4.0	250	2		
2	208	80.3	139	46.6	149	113	312	24.1	4.0	330	2		
4	314	87.1	177	63.3	178	125	318	29.7	4.0	340	2		
6	377	89.3	203	75.3	145	87	812	40.0	7.0	360	2		
7	407	94.4	201	74.5	172	91	957	47.1	7.0	360	2		
10	476	71.0	136	44.8	183	168	189	8.2	5.5	320	1½		
9	475	68.5	169	41.7	171	169	170	0.5	5.0	300	1		
5	543	78.0	138	33.3	170	165	80	3.3	6.0	300	1		
8	544*	58.0	174	49.0	162	155	86	4.3	6.0	460	½		
Av. of 2 hr. experiments											—	—	
											—	—	

* Given 0.4 c.c. 1:1000 adrenaline by constant perfusion.

the ability of cardiac muscle to use fatty acids. It is clear, however, that this power is only exercised when carbohydrate sources of energy are depleted to an extreme extent.

DISCUSSION

The relation between cardiac glycogen and blood sugar shown by our experiments with the heart-lung preparation in the rat indicates more clearly the extent to which glycogen loss is dependent upon the degree of hypoglycaemia produced during an experiment. The metabolic role of glycogen is more clearly defined in that the results of these experiments on glycogen loss indicate, in accordance with the views of many investigators, that glycogen is being continuously utilized and restored and that its loss is determined by the disappearance of circulating carbohydrate, i.e. glucose and lactic acid. Suggestions that glycogen is only used in an emergency are not borne out by these experiments.

With regard to fatty acid utilization, the question is raised as to whether or not blood fat is the source of cardiac fat. Our experiments would indicate that cardiac fat is replenished from blood fatty acids and that, while the heart can and does utilize its stored fat, the primary source of its energy in so far as it is derived from fat is the blood fatty acids. In this respect, therefore, cardiac fat as a source of energy is in the same category as cardiac glycogen, and the question of the origin of cardiac fat does not arise. It may be inferred from the results in Tables VI and VII that the loss of cardiac fat is determined by the depletion of blood fat in the same way that it is accepted that the loss of cardiac glycogen is dependent upon a greater loss of blood sugar. Further investigation may show that the loss of blood fat is related to the fall in cardiac glycogen.

While we are convinced that the mammalian heart is capable of utilizing fatty acids, we would emphasize the fact that such utilization may not be a usual physiological process. In the experiments performed the demonstration of fat utilization has only been possible in the presence of an acute shortage of carbohydrates.

SUMMARY

1. The relation of blood sugar to cardiac glycogen in the male rat has been investigated and was found to be a logarithmic one.
2. This relationship is disturbed by the addition of adrenaline to the blood perfusing the heart-lung preparation. The low cardiac glycogen produced with a comparatively high blood sugar is regarded as evidence

for a specific activity of adrenaline in causing the breakdown of cardiac glycogen.

3. The utilization of glucose and lactic acid by the heart and lungs of the rat was found to depend on their relative concentrations.

4. A perfectly viable aglycaemic heart preparation has been obtained upon which the investigation of the non-carbohydrate metabolism of the heart could be carried out.

5. It has been shown that the cholesterol and phospholipin fatty acid content of the heart is not altered by losses of blood sugar and cardiac glycogen amounting to 80 and 45 % respectively.

6. The utilization by the heart of fatty acids from the heart muscle and from the blood has been demonstrated. It has been shown that the utilization of blood and heart fatty acids depends upon the degree of depletion of carbohydrates in blood and heart muscle.

Our thanks are due to the Medical Research Council for a grant to one of us (E. W. H. C.) which defrayed the costs of this investigation, to Messrs Eli Lilly & Co. (London) for continued gifts of sodium amytal and to Mr W. Thomson of the Rowett Research Institute for the supply of rats.

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THE VASO-DILATOR ACTION OF POTASSIUM

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PREVIOUS to 1911 there was a controversy, summarized by Mathison [1911] and Regniers [1926], whether potassium caused vaso-constriction or vaso-dilatation, either in frogs or mammals. To a large extent this was concluded by Mathison, who showed that intravenous injection of potassium salts in cats caused a fall in blood pressure due to a depressant action on the heart; intra-arterial injection caused a rise of blood pressure accompanied by a decrease in limb and intestinal volume, obtainable even in the decapitate pithed animal.

However, this pressor action may not have been due to potassium itself, but to a release of adrenaline. Hazard [1933] observed that injection of potassium chloride caused an output of adrenaline from the cat's adrenals. This has subsequently been verified by Feldberg & Guimaraes [1936], by Katz & Katz [1937], and by other authors.

To discover the direct action of potassium on the vessels it is therefore necessary to use preparations in which the effect of the adrenals can be eliminated. Regniers [1926] perfused the head and legs of rabbits and cats, and the paws of dogs, with blood diluted with Ringer's solution, and observed that potassium salts always caused a vaso-constriction. Brown & Feldberg [1936] observed that potassium chloride caused a vaso-constriction in the superior cervical ganglion perfused with Ringer's solution. On the other hand, Katz & Lindner [1938], perfusing the coronary arteries of a dog with defibrinated blood, occasionally observed a brief dilatation, though more usually a prolonged constriction, and in the older literature there is similarly some evidence that potassium may cause vaso-dilatation in perfused preparations.

In order to clarify the position, investigations have been made on animals in which the adrenals have been removed, or on perfused organs;

and it has been found that potassium usually causes a vaso-dilatation in small doses, a vaso-constriction in large doses. This paper is an account of these investigations.

METHODS

The muscles of one hindleg of a dog or cat have been perfused with defibrinated blood from a Dale-Schuster pump [1928]; the venous outflow was recorded by Gaddum's recorder [1929]; the gastrocnemius was stimulated from the sciatic nerve, and its tension recorded by an isometric lever. For this preparation the animal was anaesthetized with ether, and in a few cases given chloralose, the ether being discontinued. The femoral artery and vein were then prepared and the saphenous vessels tied off; a rod was driven through the femur just above the condyles, and a length of the sciatic nerve exposed. The animal was then bled out, the blood defibrinated and passed into the Dale-Schuster pump. The lungs were perfused through the pulmonary artery, the blood being collected from a cannula in the left auricle. The leg was perfused through the femoral vessels. The tendo Achillis was freed, with a portion of the os calcis, and attached to a tension lever with a length of copper wire. A single electrode was applied to the cut sciatic nerve, the other electrode being a zinc plate wrapped in cotton, soaked in saline and placed under the skin nearby. The nerve was stimulated by condenser discharges, the frequency being determined by an oscillating valve circuit, or by a rotating commutator.

In the same way the skin vessels of a dog's hindlimb were perfused from the femoral artery, all branches of the femoral artery and vein, except the saphenous vessels, being tied off. The dog's intestine was perfused by a similar technique [Bülbring & Burn, 1936] through the superior mesenteric artery, using defibrinated blood and the lungs of the same dog to oxygenate it. The venous outflow was collected from the portal vein. The exposed coils of the gut were covered with cotton-wool soaked in warm Ringer's solution and warmed by a lamp. The lungs of a dog were perfused with defibrinated blood from the pulmonary artery, the blood being collected from a cannula in the left auricle. In this latter preparation the pressure in the arterial cannula was measured by a water manometer filled with saline and connected to a piston recorder.

Spinal cats were prepared by Dale's method. The plethysmographs used were of the type described by Bülbring & Burn [1936]. The rabbit's ear was perfused with oxygenated Ringer-Locke solution at room temperature by the method of Gaddum & Kwiatkowski [1938] the anaesthetic used being either ether or Pernoxton.

RESULTS

It has been confirmed that, in the spinal cat, adrenalectomy greatly reduces the rise of blood pressure caused by injection of potassium chloride into the carotid or iliac arteries. A similar reduction has also been obtained by clamping the adrenal veins during the period of injection; after the clamps have been removed the rise of blood pressure caused by KCl returns to its former height. The results indicate that the greater part of the rise of blood pressure caused by intra-arterial injection of KCl is due to a release of adrenaline from the adrenals. This rise of blood pressure persists after a complete nicotine paralysis, and also after removal of the splanchnic nerves and semilunar ganglia in the spinal cat. On the other hand it is abolished by ergotoxine.

In the adrenalectomized spinal cat intra-arterial injection of KCl still causes a rise in blood pressure. This varies enormously in different cats; usually 30 mg. of KCl in 5 % solution have to be injected to obtain a rise of more than 30 mm. Hg, though in exceptional cases a rise of some 70–80 mm. may be observed with the same amount. Consecutive injections usually cause progressively decreasing rises of blood pressure, and may be accompanied by widespread convulsions directly the injection is completed. Since such large quantities of KCl were required to cause a rise in blood pressure, and since the response was so variable, it seemed possible that the rise of blood pressure was a mixed effect, vaso-constriction in some parts of the body concealing vaso-dilatation in other parts. It was, for instance, interesting that in spinal or chloralose cats, either adrenalectomized or with their adrenals intact, provided that the blood pressure was above 70–80 mm. Hg, intra-arterial injection of 5–10 mg. KCl occasionally caused a fall of blood pressure of some 10–15 mm. Hg. This could not have been due to an effect upon the heart.

Vessels of the hindlimb. Plethysmograph records were taken of the hindlimb of adrenalectomized cats under chloralose. Injection was made from a catheter tied into the external iliac artery; the terminal branches of the aorta and the inferior mesenteric artery were ligated, so that all injected fluid went down the leg of the opposite side. It was found that small quantities of KCl, up to 10 mg. in 1 % solution, invariably caused an increase of limb volume with a very slight fall of blood pressure. However, if larger quantities were injected, either in 1 or 5 % solution (the latter to avoid injecting more fluid), then this dilatation was preceded by a transitory vaso-constriction (Fig. 1). Isotonic KCl is 1.12 %, so since these vascular changes have been observed with both 1 and 5 %

KCl, it is unlikely that they are due to the tonicity of the injected solutions. Moreover, 1 and 5% NaCl have almost no effect on the limb volume.

Since the animals used were adrenalectomized, these vascular reactions cannot have been due to a release of adrenaline from the adrenals. Nor is the vaso-dilator action of KCl reflex in origin, since it is readily seen in a cat under chloralose, whose femoral and sciatic nerves have been cut 4 days previously (Fig. 2).

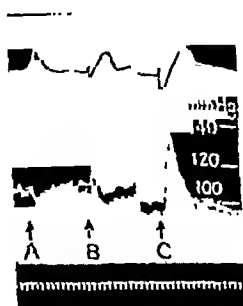


Fig. 1.

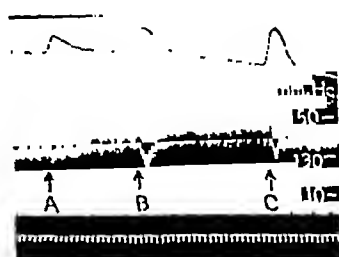


Fig. 2.

Fig. 1. Cat anaesthetized with chloralose and adrenalectomized. Upper tracing is the volume of one hindlimb; lower tracing is the blood pressure. The time tracing in this and all subsequent figures is in 10 sec. intervals. At A, injection from a catheter tied into the external iliac artery of the opposite side of 10 mg. KCl (1%) causes vaso-dilatation. At B, 10 mg. KCl (5%) causes vaso-dilatation. At C, 20 mg. KCl (5%) causes vaso-constriction.

Fig. 2. Cat anaesthetized with chloralose and adrenalectomized. Upper tracing is the volume of one hindlimb denervated 4 days previously, by cutting the femoral and sciatic nerves; lower tracing is the blood pressure. Intra-arterial injection of KCl (1%) causes vaso-dilatation. At A, 2.5 mg.; at B, 5 mg.; at C, 10 mg. KCl.

These results have been confirmed by experiments upon the perfused hindlimbs of the dog and cat. In both, the vaso-dilator and vaso-constrictor actions of KCl are seen to great advantage, since the perfusion pressure is not complicated by changes in other organs. Thus in Fig. 3 A, 5 mg. KCl has only a vaso-dilator action, 20 mg. KCl a primary constriction followed by dilatation. This is in complete agreement with the findings in the adrenalectomized chloralose cat. Moreover, in the dog's perfused hindlimb, infusion of KCl will also cause vaso-dilatation, provided that the infusion rate is less than about 100 mg. per min. At faster rates there is vaso-constriction.

If the vascular tone is raised by an infusion of adrenaline, as in Fig. 3B, the vaso-dilator action of KCl is far greater, and the vaso-constrictor action much reduced. In the same way Bülbring & Burn [1939] observed that in the dog's perfused hindlimb, muscle work caused a far greater increase of blood flow when the vascular tone was high. Finally, after injection or infusion of very large amounts of KCl (Fig. 3C),

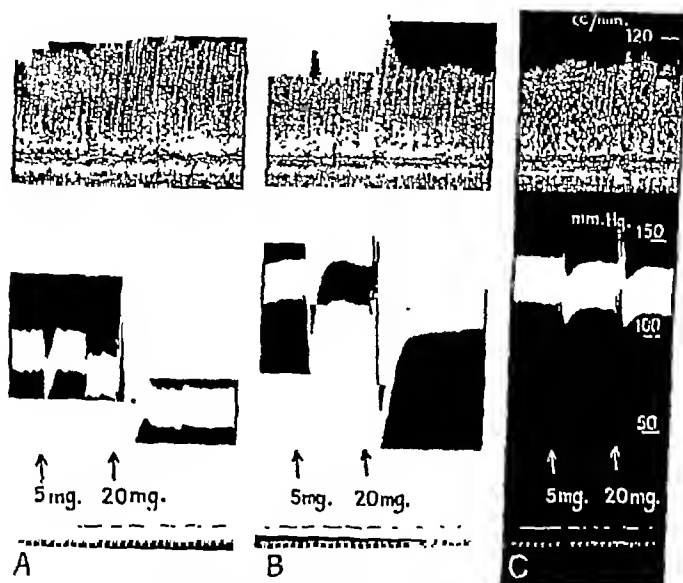


Fig. 3. The upper record shows the changes in venous outflow from the perfused muscles of a dog's hindlimb. The lower record is the pressure in the arterial cannula. A shows the effect of the injection of 5 and 20 mg. KCl at the beginning of the experiment. In B the vascular tone was raised by the infusion of adrenaline at a rate of $1.4 \mu\text{g./min.}$, and the vaso-dilatation is much greater. Between B and C, 1.05 g. KCl was infused over a total period of 30 min.; and in C the effect of KCl is reduced.

the vascular tone remains high and KCl has little action upon the vessels. Some of the reasons why the vaso-dilator action of potassium is not prominent in the intact animal may now be deduced. If the adrenals are not extirpated, the release of adrenaline on intra-arterial injection of KCl may entirely conceal the vaso-dilatation; yet if, after adrenalectomy, the vascular tone is low, then the vaso-constrictor component is more prominent, especially with larger doses. And if large amounts of KCl are injected, there is always the possibility that the vessels may become unresponsive.

Intestinal vessels. In the adrenalectomized chloralose cat KCl was injected from a catheter pushed down the left common carotid artery. The record taken by an intestinal plethysmograph showed that any vaso-dilatation there might be was small, and was partly concealed by the disturbance created by the contraction of the smooth muscle of the gut, owing to the injection of potassium.

This observation has been confirmed by results obtained in the perfused dog's intestine. Injection of 10 mg. KCl caused a barely perceptible vaso-dilatation; 40 mg. KCl caused a small rise in perfusion pressure and decrease in outflow, associated with obvious movements of the gut. Raising the vascular tone by an infusion of adrenaline did not increase these responses to any noticeable degree. The vessels of the intestine, therefore, do not react to KCl nearly as well as those of the hindlimb. Bülbring & Burn [1936] have found a similar difference with regard to acetylcholine.

Vessels of skin and lung, and spleen volume. The vessels of the skin of the dog's hindlimb, perfused through the saphenous artery, reacted to the injection of 5 mg. KCl by an increase in outflow, though there was little or no change in the pressure in the arterial cannula. A small part, therefore, of the apparent vaso-dilatation seen in the perfused dog's hindlimb, may have been due either to a dilatation of the skin vessels, or to a constriction of the skin veins, contributing to the increase in outflow.

In the perfused dog's lungs no trace of vaso-dilatation was seen. 40 mg. KCl (5 % solution) caused a rise in pressure in the arterial cannula of about 15 mm. of water and a slight increase of outflow. It seems likely that this small effect must have been due to the contraction of smooth muscle in the lungs.

The reaction of the spleen to KCl was also observed, by passing the spleen through a slit in the abdominal wall, and recording its volume from a plethysmograph. Injection of up to 10 mg. KCl from a carotid catheter in the adrenalectomized chloralose or spinal cat had no effect upon the spleen volume. It was concluded that potassium was more likely to cause contraction than relaxation of the spleen in the whole animal, owing to the liberation of adrenaline.

The isolated perfused rabbit's ear. In this preparation KCl only causes vaso-constriction. This falls into line with the report of Brown & Feldberg [1936], that KCl causes vaso-constriction in the superior cervical ganglion perfused with Locke's solution. Similarly, in the rat's hindlegs perfused at room temperature from the aorta with Locke's solution from a Marriotte bottle, KCl causes only vaso-constriction. These preparations

differ from the normal in that the oxygen supply is poor and the temperature low; and it is probable that the explanation for the unusual vascular reactions lies in these differences.

Action of KCl on adrenaline vaso-constriction. Bülbring & Burn [1939] observed that the vaso-dilatation produced by working muscles was much greater when the vascular tone was raised by an infusion of

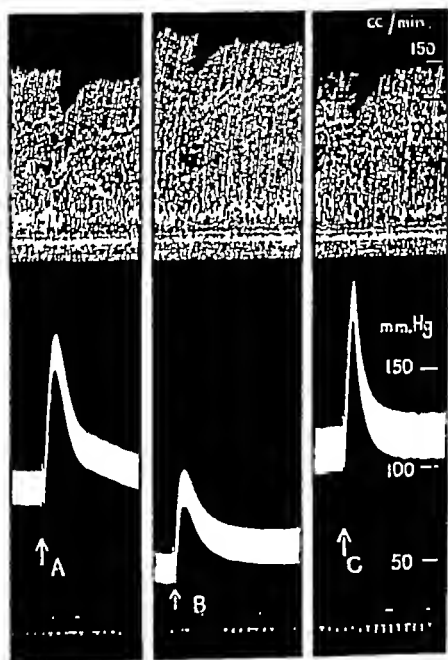


Fig. 4. Records as in Fig. 3. Injections of $4 \mu\text{g}$. adrenaline, A before, B during and C after the infusion of KCl at a rate of 84 mg. per min. The increase of arterial resistance due to the adrenaline was 60 mm. during the infusion, as compared to 90 mm. before and 100 mm. afterwards.

adrenaline, and a similar observation has been made for potassium (Fig. 3). They also showed, in confirmation of Mertens, Rein & Valdecasas [1935], that injection of adrenaline during muscular work had less constrictor effect than before or after work. In Fig. 4 it will be seen that adrenaline has a smaller vaso-constrictor action during an infusion of KCl, at a rate sufficient to lower the vascular tone considerably.

However, this is not a phenomenon peculiar to potassium ions. Infusion of histamine causes a fall of vascular tone, and injection of

adrenaline may then have either a decreased or an increased vaso-constrictor effect. A slow infusion of acetylcholine with a similar fall of tone predisposes to an increase in the action of adrenaline; but if the infusion rate be sufficiently great, the vaso-constriction caused by adrenaline may be reduced.

The action of other ions. In the perfused dog's hindlimb injection of 10 mg. KCl (1 %) has a rather greater vaso-dilator effect than 10 mg. KCl (5 %). CaCl_2 causes a pronounced vaso-dilatation in 1 or 5 % solution, though not so large as that produced by KCl (Fig. 5). On the other hand,

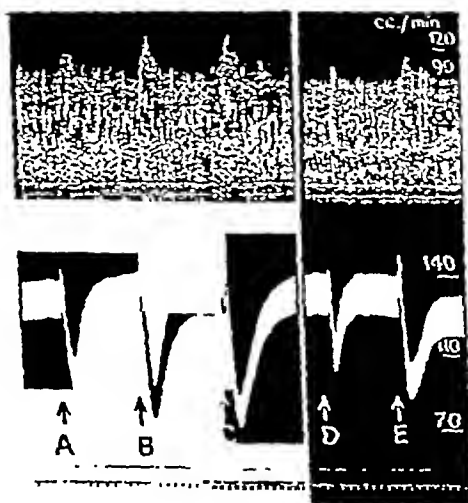


Fig. 5. Records as in Fig. 3. At A injection of 10 mg. KCl (5%); at B, 10 mg. KCl (5%) + 40 mg. CaCl_2 (5%); at C, 40 mg. KCl (5%). At D injection of 5 mg. CaCl_2 (5%); at E, 40 mg. CaCl_2 (5%). The vaso-dilatation caused by KCl is not antagonized by, and is greater than that of, calcium.

NaCl (1 %) has no vascular action, a 5 % solution causes a slight vaso-dilatation, much less conspicuous than that of KCl or CaCl_2 .

In view of this it was interesting to observe the effect of CaCl_2 upon the vaso-dilatation caused by KCl. In many other organs the antagonism between the two ions is well established; but it is also true that the individual actions of the ions are in these cases in opposite directions. Thus potassium causes a contraction of isolated strips of gut, calcium a relaxation. It is not surprising that in such cases an antagonism is observed. On the other hand, Tate & Clark [1922] state that in the isolated non-pregnant uterus of the rabbit and cat, in which both KCl and CaCl_2 cause contraction, there is no such antagonism.

In the perfused hindlimb of the dog or cat the vaso-dilator actions of KCl and CaCl_2 appear to be purely additive. In the experiment shown in Fig. 5, the injection of 10 mg. KCl + 40 mg. CaCl_2 caused a fall in arterial pressure and an increase in outflow equalled by the injection of 40 mg. KCl. This contrasts strikingly with the finding that, in the spinal cat with or without adrenals, intra-arterial injection of 40 mg. KCl + 40 mg. CaCl_2 caused less than half the rise of blood pressure given by KCl alone; and this in spite of the fact that according to Katz & Katz [1937] CaCl_2 also releases adrenaline from the adrenals.

The mode of action of potassium. In the perfused hindlimbs of the cat and dog sufficient atropine was given to abolish completely the vaso-dilator action of acetylcholine. The vaso-dilatation caused by KCl, CaCl_2 and muscular work was not reduced. Therefore, though Brown, Feldberg and others have observed a liberation of acetylcholine by potassium in a number of preparations, it is improbable that the vaso-dilatation caused by KCl is produced in this way.

The vaso-constrictor action of potassium is a more complex problem. Since KCl has been observed to cause a liberation of adrenaline from the adrenals, and since Brown & McIntosh [1939] have found that KCl may stimulate nerve fibres in continuity, it was possible that part of the vaso-constriction caused by KCl was due to the stimulation of adrenergic nerves, post-ganglionic sympathetic fibres, for instance, causing a release of adrenaline or sympathin.

This possibility was supported by the fact that in the spinal cat, with intact adrenals or with its adrenals extirpated, the rise of blood pressure caused by intra-arterial injection of KCl is almost entirely abolished by ergotoxine (Fig. 6). Moreover, in the spinal adrenalectomized cat, ephedrine has occasionally been seen to increase the rise of blood pressure caused by KCl. Similarly, in the perfused rabbit's ear, ephedrine sometimes potentiates the vaso-constriction caused by KCl.

In addition, injection of potassium may cause a rise of blood pressure, liable to interpretation as vaso-constriction, owing to muscular contrac-

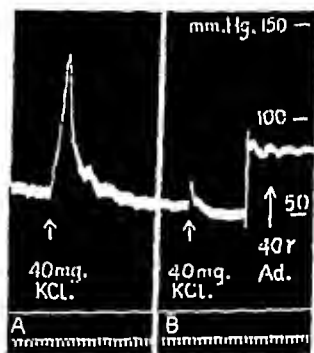


Fig. 6. Spinal cat adrenalectomized. Injections of 40 mg. KCl, from a catheter passed down the left carotid artery. Between A and B, 7 mg. ergotoxine was injected intravenously. In this particular preparation, although the vaso-constrictor action of adrenaline was abolished, there was no reversal. The rise of blood pressure due to KCl is reduced.

tion. It is well known that direct application of KCl causes both smooth and striated muscle to contract, and this effect may be supplemented by the excitation of motor nerves. In the spinal cat injection of KCl in sufficient amounts to cause a reasonably large rise of blood pressure evokes widespread muscular twitches. These twitches are less prominent during a nicotine paralysis, possibly owing to a depression of muscular excitability. Similarly, in the dog's perfused intestine, injection of KCl in sufficient amounts to cause a rise of 15–20 mm. Hg in the perfusion pressure caused very considerable movements of the gut. There are, therefore, at least three possible factors concerned in the rise of blood pressure caused by KCl in the adrenalectomized spinal cat, namely, a direct constrictor action on the vessels, the stimulation of vaso-constrictor nerves, and the contraction of muscles.

The action of KCl upon contracting muscle. It is well known that injection of an excess of KCl will depress the muscle twitch evoked by a single shock applied to the nerve; smaller amounts of KCl have also been shown to potentiate twitches by Baetjer [1935], Wilson & Wright [1936] and others. Bülbring & Burn [1939] have shown that when the blood flow is small in perfusion experiments, the tension developed during a tetanus can be increased by an increase in blood flow, caused by raising the pump stroke. Since it had been found that KCl would cause vaso-dilatation in small doses, vaso-constriction in large doses, the possibility arose that the muscle changes might be dependent upon the vascular changes. Investigations were undertaken on the perfused dog's hindlimb to test this possibility.

Three types of stimulation were applied to the sciatic nerve, single shocks at a rate of one every 4 sec., interrupted stimuli at 78 per min., and tetani at 460 per sec. It was found that injection of KCl potentiated the response to single shocks, whether the injection was accompanied merely by vaso-dilatation, or as in Fig. 7 A by vaso-constriction followed by dilatation. Thus during single shocks there was no direct relation between the vascular and the muscular changes. During interrupted stimuli, as in Fig. 8, a large vaso-constriction was always accompanied by a fall in the tension developed; a vaso-dilatation was not necessarily related to a rise in tension. During tetani, as in Figs. 7 B and 9, a vaso-constriction was associated with a decrease in tension, and a vaso-dilatation was only sometimes related to an increase. Moreover, during a tetanus, as in Fig. 9 B, vaso-dilatation was sometimes associated with a fall in tension; and during interrupted stimuli a rise of tension has been seen to follow the injection of KCl without any trace of vaso-dilatation.

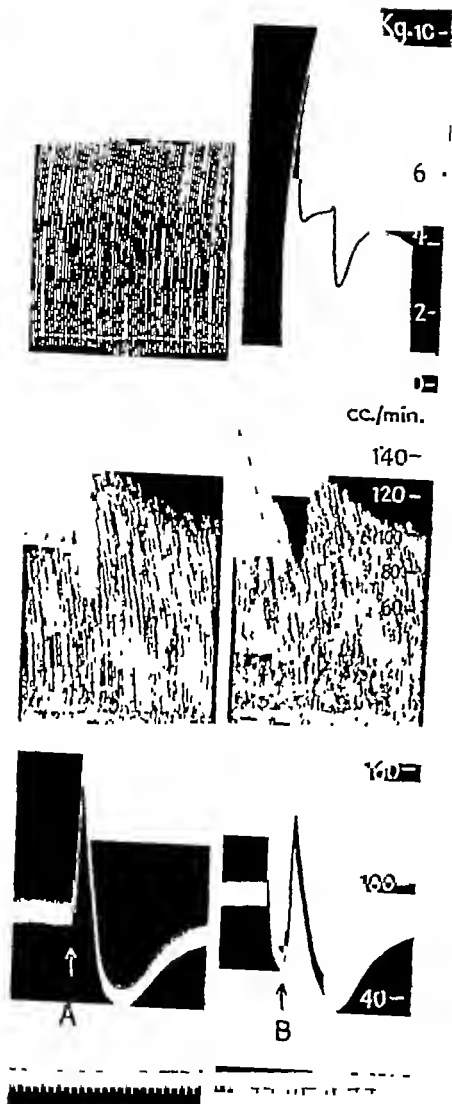


Fig. 7.

Fig. 7. The upper record shows the tension in the gastrocnemius muscle of a perfused dog's hindlimb; the middle record is the venous outflow; the lower record is the pressure in the arterial cannula. Injections of 40 mg. KCl were made, at A during single shocks applied to the sciatic nerve at a rate of 15 per min., and at B during a tetanus at a rate of 460 per sec. The same amount of KCl which causes an increase in the tension developed during single shocks causes a decrease during a tetanus.

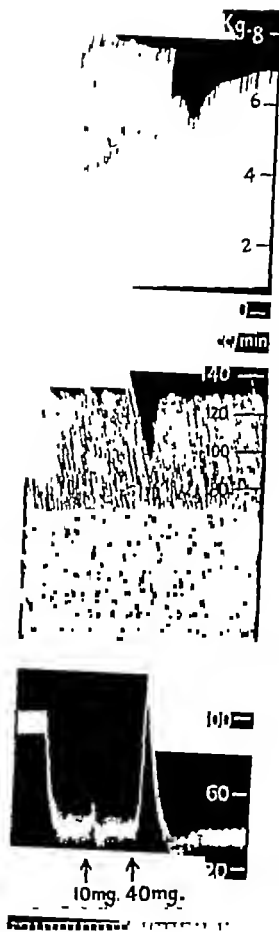


Fig. 8.

Fig. 8. The gastrocnemius was stimulated by interrupted stimuli applied to the sciatic nerve at a rate of 78 per min. Injection of 10 mg. KCl had little vascular or muscular effect; 40 mg. KCl caused a pronounced vaso-constriction, accompanied by a reduction in the tension developed.

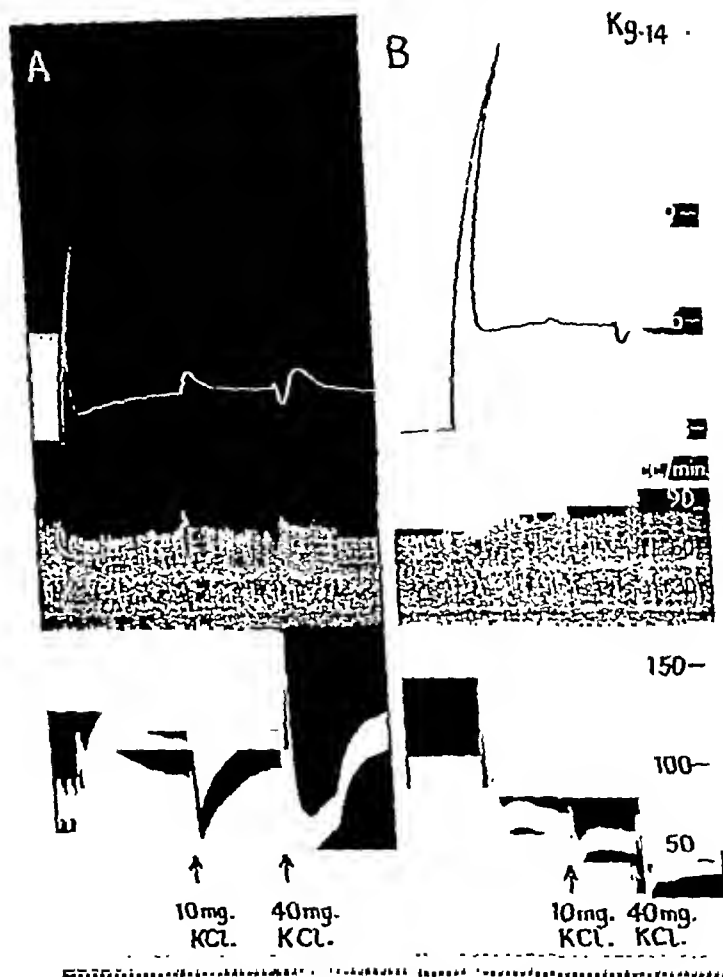


Fig. 9. In A the gastrocnemius was fatigued by the application of interrupted stimuli to the sciatic nerve, and was then subjected to a tetanus. Injection of 10 mg. KCl caused a vaso-dilatation and an increase in the tension developed; 40 mg. KCl caused a primary vaso-constriction accompanied by a fall in tension. Between A and B the muscle was allowed to rest for 15 min. 10 mg. KCl then had almost no effect on the tension produced during a tetanus, and 40 mg. KCl caused a vaso-dilatation accompanied by a reduction in tension.

It is therefore clear that the depressant and stimulant action of KCl upon active muscle is not primarily dependent upon its vascular action. Nevertheless, it is believed that under certain circumstances there

may be a fairly close relation between the muscular and vascular effects.

During tetani or interrupted stimuli a vaso-constriction after the injection of KCl always causes a fall in tension, whereas during single shocks it does not necessarily do so; in fact, the greater the frequency of stimulation, the closer the correspondence between the vascular and muscular reactions. This suggests that, as the amount of work done by a muscle increases, the "safety margin" between actual blood flow and blood flow required to maintain the contractions diminishes. Thus, when the muscle is working hard, or becomes fatigued, any reduction in blood flow will be accompanied by a fall in the tension developed. In Fig. 9A a muscle had been fatigued by interrupted stimuli immediately before a tetanus; in Fig. 9B the muscle was allowed to recover, and in consequence the tension produced was far greater. In A, when the muscle is fatigued, the vascular effects are strikingly allied to the muscular, whereas in B the relation is not so clear.

DISCUSSION

The experiments clear up some of the contradictions in the earlier literature about the vascular action of potassium. According as the dose of KCl is small or large, the vascular tone high or low, the oxygenation good or bad, KCl may cause vaso-dilatation or constriction. The vaso-dilatation is essentially a peripheral effect, and is best seen in the perfused hindlimbs. Other effects of KCl are to cause a general rise of blood pressure by the release of adrenaline, and thereby to cause the spleen to contract and the intestinal vessels to constrict. The amount of KCl required to produce these changes is relatively small and within the physiological range; the total effect of a release of potassium from, for instance, the hindlimbs, would be to cause abdominal constriction and peripheral dilatation, thus increasing the blood flow through the limbs.

It is well known that during or directly after muscular work the blood flow through the muscles is greatly increased, and this has been ascribed to the influence of some metabolite produced during work and released into the blood stream. Anrep & Saalfeld [1935] have shown that this is not acetylcholine, since the hyperaemia is unaffected by eserine and atropine. I have been able to confirm the fact that atropine, sufficient to abolish the vaso-dilatation caused by acetylcholine, does not affect the vaso-dilatation caused by muscular work. Anrep further reports that in dogs the vaso-dilator substance did not deteriorate when kept in blood for 20-30 min., or in contact with the tissues. Similarly, Grant &

Pearson [1937] have shown in man that it was not destroyed by obstruction of the circulation for 7 min. It would therefore appear unlikely that this vaso-dilator substance is adenosine, since Drury, Lutwak-Mann & Solandt [1938] have shown that there is an enzyme system in blood which destroys adenosine. Anrep, Barsoum, Talaat & Wieninger [1939] have confirmed their observations that muscular contractions release histamine; thus histamine, if it is not destroyed by prolonged contact with the tissues, may be the vaso-dilator substance.

There is no doubt that potassium ions are not destroyed by the tissues; and there is considerable evidence that they are released during muscular work. Thus Ernst & Fricker [1934] have described an increase in the amount of diffusible potassium in muscle after prolonged activity. Fenn [1939] has demonstrated a fall in the potassium content of the muscles of cats, rats and frogs after work. Baetjer [1935] has observed an increase in the potassium content of the blood coming from muscles stimulated from the anterior spinal roots. Moreover, it has been suggested by Brown & von Euler [1938] and others that release of potassium ions is responsible for post-tetanic potentiation. If sufficient potassium is released during muscular activity to cause post-tetanic potentiation, it seems very probable that sufficient is released to cause vaso-dilatation. Moreover, the action of KCl in causing a greater vaso-dilatation when the vascular tone is high, and in reducing the constrictor effect of adrenaline injected into the perfused hindleg of a dog, is very similar to the phenomenon observed during muscular activity, and described by Mertens *et al.* [1935] and Bülbring & Burn [1939]. It therefore seems probable that potassium is one of the factors concerned in the hyperaemia of muscular work.

Little light is thrown by these experiments on the mechanism whereby KCl potentiates or depresses muscle, since both potentiation and depression have been observed under conditions in which they could not be attributed to the vascular changes. On the other hand, since such changes undoubtedly play an important part in the tension produced by a working muscle, they cannot be disregarded altogether, and it will be necessary to eliminate them in any experiments undertaken to elucidate this mechanism.

SUMMARY

1. Small doses of KCl cause vaso-dilatation, larger doses vaso-constriction, in the hindlimbs of adrenalectomized cats under chloralose, or in the perfused hindlimbs of dogs and cats. The vaso-dilatation is not affected by atropine, nor antagonized by calcium.

2.. KCl also causes an increased outflow from perfused skin vessels of the dog's hindlimb, but has no effect on lung vessels, and very little on intestinal vessels.

3. The rise of blood pressure caused by intra-arterial injection of KCl in the adrenalectomized spinal cat is greatly reduced by ergotoxine, and may occasionally be increased by ephedrine.

4. The vaso-constriction caused by adrenaline in the dog's perfused hindlimb is reduced by an infusion of KCl; this reaction is not, however, peculiar to potassium salts.

5. The potentiation and depression of working muscle by KCl is not necessarily dependent upon the vascular changes caused by KCl. However, the greater the frequency of the stimuli and the more fatigued the muscle becomes, the closer is the relation between the muscular and vascular changes.

6. The possibility is discussed that the release of potassium ions is the cause of the vaso-dilatation which occurs in contracting muscle.

I wish to express my thanks to Prof. Burn for his constant advice and encouragement, and to Mr H. W. Ling for his help with the experiments.

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THE ABSORPTION OF PENTOSE FROM THE SMALL INTESTINE OF THE RAT UNDER URETHANE ANAESTHESIA

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SUCH hexoses as glucose and galactose are known to be absorbed from the small intestine of the rat more rapidly than the pentoses xylose and arabinose [Davidson & Garry, 1940]. These hexoses, moreover, are known to be capable of phosphorylation *in vivo*, while there is no evidence for a similar process with xylose and arabinose. Such findings, along with other evidence, suggest that the absorption of glucose and galactose is accelerated by phosphorylation, while the absorption of the pentoses depends on simple diffusion [Verzár & McDougall, 1936].

Wilbrandt & Laszt [1933] found that xylose and arabinose are absorbed relatively slowly and at the same rate from the intestine of the rat. Cori [1925], on the other hand, found that xylose was absorbed more rapidly than arabinose by the rat. Similar observations were made by McCance & Madders [1930] in human beings, by Westenbrink [1936] in the rat and pigeon, and by Westenbrink & Gratama [1937] in the frog. Westenbrink alone [1936] compared the two optically active forms of the same sugar, finding that the pigeon absorbed the naturally occurring *d*(+)-xylose more rapidly than *l*(-)-xylose.

We have extended these observations in the rat, using the *d* and *l* forms of both xylose and arabinose. We also used the naturally occurring *d*(-)-ribose whose absorption rate has not so far been determined, although it is present in phosphorylated form as a constituent of the ribo-nucleic acid derivatives.

We adapted the two-loop technique, already described for cats, to the rat [Davidson & Garry, 1940]. In one series the *d* form was placed in the cranial loop, and the *l* form of the same sugar in the caudal loop. In the other series the positions of the optical isomers were reversed. When

d(-)-ribose was used in one loop, xylose, in default of the optical isomer of *d*(-)-ribose, was present in the other loop.

1.5 c.c. of a 4.5% solution of the sugar was run into each loop and the amount remaining after 90 min. estimated. The sugars used were *d*(+)-xylose ($[\alpha]_D+18.3^\circ$), *l*(-)-xylose ($[\alpha]_D-19.3^\circ$), *d*(-)-arabinose ($[\alpha]_D-108^\circ$) and *l*(+)-arabinose ($[\alpha]_D+106^\circ$), all supplied by Roche Products, Ltd. *d*(-)-Ribose was prepared from guanosine by the method of Levene & Clark (1921) and had $[\alpha]_D-19.9^\circ$.

The averages of the results obtained from several experiments in the case of each sugar are as follows:

	Mg. absorbed in 90 min. per g. gut
<i>d</i> (+)-Xylose	17.3
<i>l</i> (-)-Xylose	10.1
<i>d</i> (-)-Arabinose	9.5
<i>l</i> (+)-Arabinose	8.6
<i>d</i> (-)-Ribose	10.7

The absorption rate of any one sugar was essentially the same whether it was in the cranial or caudal loop.

In agreement with the findings of the majority of other workers, *d*(+)-xylose was absorbed more rapidly than arabinose. In addition, the rat absorbed *d*(+)-xylose more rapidly than its optical isomer, as was found by Westenbrink for the pigeon. The other four sugars, however, *l*(-)-xylose, *d*(-)-arabinose, *l*(+)-arabinose and *d*(-)-ribose, were absorbed at about the same rate. It thus appears that there must be some special property of *d*(+)-xylose which renders it more susceptible to absorption than the other pentoses. It is known, too, that *d*(+)-xylose is absorbed, rather unexpectedly, as rapidly as glucose from the small intestine of the cat [Davidson & Garry, 1940]. It may be that some special significance attaches to the fact that *d*(+)-xylose is the only pentose to have the same configuration as *d*(+)-glucose at carbon atoms 2, 3 and 4.

Our thanks are due to the Carnegie Trust for the Universities of Scotland for an expenses grant.

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THE DISTRIBUTION OF THE PITUITARY ANTIDIURETIC HORMONE THROUGHOUT THE VERTEBRATE SERIES

By H. HELLER¹

From the Department of Pharmacology, Oxford

(Received 19 August 1940)

BURGESS, HARVEY AND MARSHALL stated in 1933 that the antidiuretic hormone induces accelerated reabsorption in the avian and mammalian kidney only. Since the thin segment of the loop of Henle is present in the kidney of these classes of vertebrates only, they assumed that the thin segment is the site of the hormone's action. Gersh's [1934] histochemical observations on the site of water reabsorption in rats and rabbits supported this hypothesis.

Is there then a correlation between the phylogenetic development of the antidiuretic hormone of the posterior pituitary gland and the development of Henle's loop in the vertebrate kidney? Does the pituitary gland of lower vertebrates (on which extract of mammalian pituitary glands are reported to have no antidiuretic action) produce a principle identical with the antidiuretic hormone of the mammal? The posterior lobe, or, to use the more appropriate term, the pars nervosa of the pituitary gland of some lower vertebrates (teleost fishes, amphibians, reptiles), has been shown to contain a pressor principle [Herring, 1913], but no demonstration of the antidiuretic hormone, whether qualitative or quantitative, seems to have been attempted. Even data on the antidiuretic hormone content in higher vertebrates, i.e. birds or mammals, are very meagre. Quantitative determinations of the antidiuretic hormone of the pars nervosa of the pituitary gland seem to be recorded for human beings [Lampe, 1926] and cats only [Fisher, Ingram & Ranson, 1938]. The presence of a pituitary antidiuretic principle in birds has been noted by De Lawder, Tarr & Geiling [1934].

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from the anaesthetic. The blood histamine in all animals which had received cortical extract after adrenalectomy was decreased 6 hr. after adrenalectomy (Table III).

TABLE III. The effect of cortical extract on blood histamine
6 hr. after adrenalectomy

Animal no.	Blood histamine ($\mu\text{g./c.c.}$)		
	Before operation	After operation	% difference
38	4.30	2.3	-46.5
39	2.90	1.8	-37.9
41	2.23	1.7	-23.8
42	1.65	1.6	-3.0
44	1.60	1.3	-18.8

DISCUSSION

Much attention has been paid to the protective mechanism against the action of histamine. The inactivation of histamine by the enzyme histaminase has been studied by Best & McHenry [1930] and others. On the other hand, Anrep, Barsoum & Talaat [1936], following the observations of Weiss, Ellis & Robb [1929], and of Jacobs & Mason [1936], have shown that "when histamine is given by slow intravenous injection to dogs it does not remain in active form in the plasma". They stated that the histamine is, in part, rendered physiologically inactive by being taken up by the red blood cells. Later, Code [1938] pointed out that, while the white blood cells carry the major part of the blood histamine during anaphylactic shock in dogs, the plasma contains the greater part of histamine at the peak of reaction. In their experiments with rats, Rose & Browne [1938] made interesting comparisons on the fate of intravenously injected histamine in normal and adrenalectomized animals. They found that at the end of 3 hr. the amount of histamine in the blood of the normal rat was $0.2 \mu\text{g./c.c.}$, whereas in the adrenalectomized animal it was $7.0 \mu\text{g./c.c.}$ While they stated that the initial blood histamine level in the normal rat is $0.5 \mu\text{g./c.c.}$, they do not appear to have determined the corresponding level in the adrenalectomized animal. The evidence produced by the experiments here described indicates that an explanation of the decrease in the rate of disappearance of histamine injected into the adrenalectomized rats may be due in part to an increase in the initial blood histamine level. The use of cortical extract in the treatment of "shock" has been advocated by Swingle, Parkins, Taylor & Hays [1936] and that in circulatory collapse the cortical hormone exerts the control of capillary tone. Moon [1939] has pro-

vided interesting evidence of the pathological changes in viscera in burns, and has pointed out the haemorrhagic appearance of the suprarenal glands in such cases. He suggested the use of cortical extract in the treatment of burns. Barsoum & Gaddum [1936] have shown that in burns the blood histamine is increased, and suggested that this increase may be secondary to pathological changes in organs such as the liver and kidneys. The experiments here described suggest the use of cortical extract where the blood histamine is raised, and further that the cortical extract should be administered as soon as possible. It is realized that estimations of plasma histamine as well as whole blood histamine are desirable but, as Code [1937] points out, there are technical difficulties associated with the separation of plasma in rabbit's blood. Investigations are proceeding on the effect of cortical extract on whole blood and plasma histamine in cases of burning and in Addison's Disease.

SUMMARY

Adrenalectomy produces a rise in the blood histamine of rabbits. Cortical extract given subcutaneously reduces the blood histamine level of adrenalectomized rabbits within 1 hr. This effect is maintained for a period of from 3 to 24 hr.

I wish to express my gratitude to Prof. J. H. Gaddum for his interest and valuable advice. It is a pleasure to acknowledge my thanks to Dr F. Schütz for adrenalectomizing the rabbits in the earlier part of the work and to Mr E. Salvin for technical assistance. I am indebted to Messrs Organon Laboratories for supplies of cortical extract used in these experiments.

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THE DISTRIBUTION OF THE PITUITARY ANTIDIURETIC HORMONE THROUGHOUT THE VERTEBRATE SERIES

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BURGESS, HARVEY AND MARSHALL stated in 1933 that the antidiuretic hormone induces accelerated reabsorption in the avian and mammalian kidney only. Since the thin segment of the loop of Henle is present in the kidney of these classes of vertebrates only, they assumed that the thin segment is the site of the hormone's action. Gersh's [1934] histochemical observations on the site of water reabsorption in rats and rabbits supported this hypothesis.

Is there then a correlation between the phylogenetic development of the antidiuretic hormone of the posterior pituitary gland and the development of Henle's loop in the vertebrate kidney? Does the pituitary gland of lower vertebrates (on which extract of mammalian pituitary glands are reported to have no antidiuretic action) produce a principle identical with the antidiuretic hormone of the mammal? The posterior lobe, or, to use the more appropriate term, the pars nervosa of the pituitary gland of some lower vertebrates (teleost fishes, amphibians, reptiles), has been shown to contain a pressor principle [Herring, 1913], but no demonstration of the antidiuretic hormone, whether qualitative or quantitative, seems to have been attempted. Even data on the antidiuretic hormone content in higher vertebrates, i.e. birds or mammals, are very meagre. Quantitative determinations of the antidiuretic hormone of the pars nervosa of the pituitary gland seem to be recorded for human beings [Lampe, 1926] and cats only [Fisher, Ingram & Ranson, 1938]. The presence of a pituitary antidiuretic principle in birds has been noted by De Lawder, Tarr & Geiling [1934].

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The present series of experiments consists of quantitative determinations of the antidiuretic activity of pituitary glands of representatives of the following classes of vertebrates: mammals, birds, amphibians and fishes. In addition, an attempt has been made to compare the antidiuretic and pressor activity of such extracts. This seemed of particular interest as it has recently been shown [Heller, 1939, 1940] that a non-pressor but antidiuretic extract can be prepared from the posterior lobe of mammalian glands. The finding of a significant disproportion between the amounts of the two principles would be further evidence that these two substances are not identical.

METHODS

The choice of animals belonging to the various vertebrate classes was limited by the circumstances. Reptiles were unobtainable. All animals with the exception of the fishes were killed by decapitation. Since fish could not be obtained living, the pituitary glands of dead fishes which had been kept in a refrigerator for various lengths of time were used. The whole pituitary glands and the parts of the brain proximal to the gland were used for the preparation of extracts. The loss of active material in structures outside the hypophysis proper is thus excluded.

Extracts were prepared by mashing the material with 1.0 c.c. of 0.25 % acetic acid in 0.9 or 0.6 % saline and boiling for 6 min. The extract was then filtered and the residue washed twice with 1.0 c.c. of saline. The filtrate consisted of a clear colourless fluid. The extracts were assayed on the day of preparation. Extracts of approximately equal amounts of indifferent brain tissue prepared in an identical manner were used for control observations.

Methods of assay. Spinal cats were used for the estimation of pressor potency. Two methods were used for the antidiuretic assay. (1) Subcutaneous injection into rats [Burn, 1931]; this method, though quite satisfactory for assays of pure extracts, is open to the objection that impurities have been shown to retard, inhibit or in certain cases, augment the action of the posterior pituitary antidiuretic principle [Heller & Urban, 1935; Noble, Rinderknecht & Williams, 1939] presumably by changing the rate of absorption from the subcutaneous tissue. Estimations were therefore made by (2) intravenous injections into rabbits. Rabbits weighing 1.6–2.5 kg. were kept on a standard diet of bran, oats and cabbage. At the beginning of the experiment they were given 5 % of their body weight of tepid water by stomach tube. A second and if

necessary a third dose of water was given later. The antidiuretic hormone content of samples of unknown strengths was estimated in terms of doses of pure pitressin (Parke, Davis and Co.). The rabbits were first tested by injections of 1 milliunit (mU.)¹ of pitressin per rabbit. A series of dilutions of the unknown samples was prepared and matched with the response to the injection of pure pitressin. The method has the advantage of great sensitivity, the rabbit having been shown to react to as little as 0.5 mU. of the antidiuretic principle per animal [Heller, 1937; Walker, 1939]. A method of such sensitivity proved to be essential for the estimation of the antidiuretic hormone content of the glands of certain small animals. The method has the disadvantage that the water diuresis of some rabbits is sometimes inhibited spontaneously. However, these very occasional interruptions have never lasted longer than 30 min. and were of a different magnitude from those caused by the injection of 1.0 mU. of pure pitressin. This disadvantage was further reduced by the use in the same experiment of several (usually three) rabbits injected with doses of the same extract. The method, while not as accurate as that of Burn, when used for a pure preparation, yielded as satisfactory results as those obtained by Walker [1939]. It should be added that an antidiuretic action of the control extracts prepared from brain samples of each species of animal was in no case observed.

RESULTS

The antidiuretic hormone content of the pituitary glands of various classes of vertebrates

Preliminary "range-finding" experiments are not quoted. Experiments shown in figures are not included in the tables.

A. *Mammals* (Fig. 1, Tables I and II).

Fisher *et al.* found approximately 10 units of antidiuretic activity in the posterior pituitary lobe of the cat. This gives an antidiuretic hormone content of 400 mU. per 100 g. body weight if 2.5 kg. is assumed as the average weight of their cats. This figure is of the same order of magnitude as those shown for the rats and mice in the present series (Table VIII).

B. *Birds* (Fig. 2, Tables III and IV).

C. *Amphibians* (Fig. 3, Table V).

An attempt was made to estimate the antidiuretic potency of frog pituitary extracts by Burn's method of subcutaneous injections into rats. However, control experiments with extracts of indifferent brain tissue

¹ The term milliunit (mU.) is used to denote the activity of 0.0001 c.c. of pituitary (posterior lobe) B.P. extract.

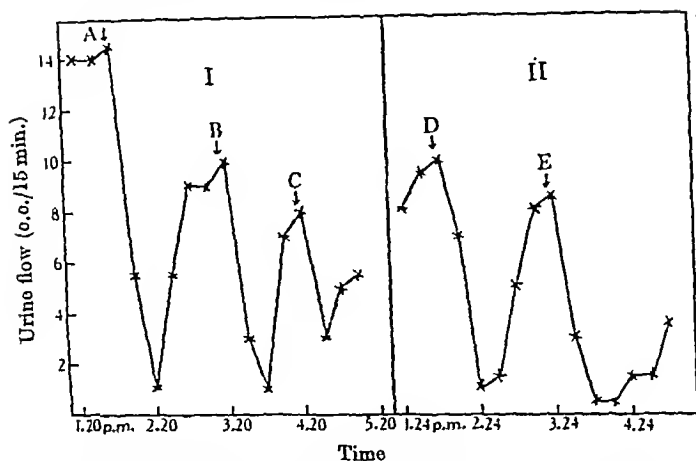


Fig. 1. Estimation of antidiuretic hormone content of a *rat* pituitary gland. Rat no. 6, 315 g. ♂. Extract of pituitary gland diluted to 1000 c.c. I=rabbit no. 56. At 10.23 a.m. and at 12.30 p.m. 5 % of body weight of water by stomach tube. A=1.0 c.c. of rat pituitary extract i.v., B=1.0 mU. pitressin i.v., C=0.8 c.c. of rat pituitary extract i.v. II=rabbit no. 19. At 10.27 a.m. and at 12.32 p.m. 5 % of body weight of water. D=1.0 mU. pitressin i.v., E=1.25 c.c. of rat pituitary extract i.v. It will be noted that 0.8 c.c. of the rat pituitary extract (C) had a smaller and 1.25 c.c. (E) a greater inhibitory effect on the water diuresis than 1.0 mU. pitressin (B and D). The antidiuretic activity of the rat pituitary extract was equivalent, therefore, to less than 1250 and more than 800 mU. pitressin. 1.0 c.c. of the extract (A) has much the same effect as 1.0 mU. pitressin (B) indicating that the rat pituitary extract contained approximately 1000 mU. of an antidiuretic principle. A third rabbit was used in the same experiment and gave essentially similar results.

TABLE I. The antidiuretic hormone content of *rat* pituitary glands. For description of method of estimation of antidiuretic potency see "Methods" and legend of Fig. 1

No.	Sex	Weight of animal in g.	Antidiuretic hormone content of pituitary gland in milliunits pitressin	
1	♂	370	> 500	<1000
2	♂	320	> 800	<1200
3	♂	271	>1000	<1200
4	♂	277	>1200	<1500
5	♂	260	> 800	<1000

TABLE II. The antidiuretic hormone content of *mouse* pituitary glands

No.	Sex	Weight of animal in g.	Antidiuretic hormone content of pituitary gland in milliunits pitressin	
1	♂	17.2	>20	<40
2	♂	20.5	>30	<50
3	♂	19.7	>30	<50
4	♂	20.5	>30	<60
5	♂	18.4	>30	
6	♂	27.9	>20	<40
7	♂	21.4	>30	<50

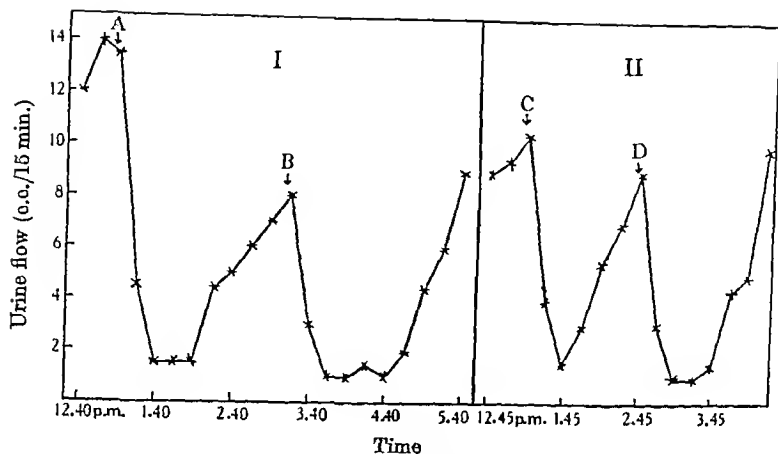


Fig. 2. Estimation of antidiuretic hormone content of a pigeon pituitary gland. Pigeon no. 13, 445 g. ♀. Extract of pituitary gland diluted to 20.0 c.c. I=rabbit no. 52. At 10.18 a.m. and 12.00 p.m. 5 % of body weight of water by stomach tube. A=1.0 mU. pitressin i.v., B=1.0 c.c. of pigeon pituitary extract i.v. II=rabbit no. 72. At 10.21 a.m. and 12.04 p.m. 5 % of body weight of water. C=0.5 c.c. of pigeon pituitary extract i.v., D=1.0 mU. pitressin i.v. 1.0 c.c. of the pigeon pituitary extract had a greater and 0.5 c.c. a smaller inhibitory effect on the water diuresis than 1.0 mU. of pitressin. The antidiuretic activity of the extract was, therefore, equivalent to more than 20 and less than 40 mU. pitressin. A third rabbit, injected with doses of the same extract, gave essentially similar results.

TABLE III. The antidiuretic hormone content of pigeon pituitary glands

No.	Sex	Weight of animal in g.	Antidiuretic hormone content of pituitary gland in milliunits pitressin	
1	♂	415	>25	<50
2	♂	487	>20	<40
3	♂	436		<50
4	♂	474	>20	<40
5	♂	381	>20	<40
6	♂	434	>20	<40

TABLE IV. The antidiuretic hormone content of drake pituitary glands

No.	Weight of animal in g.	Antidiuretic hormone content of pituitary gland in milliunits pitressin	
1	1850	>80	<160
2	1800	>70	<100
3	1930	>50	<80

were not satisfactory, and the responses to injections of pituitary extracts when compared with those obtained by the rabbit method indicated a much higher antidiuretic potency (Table V, Exp. 1).

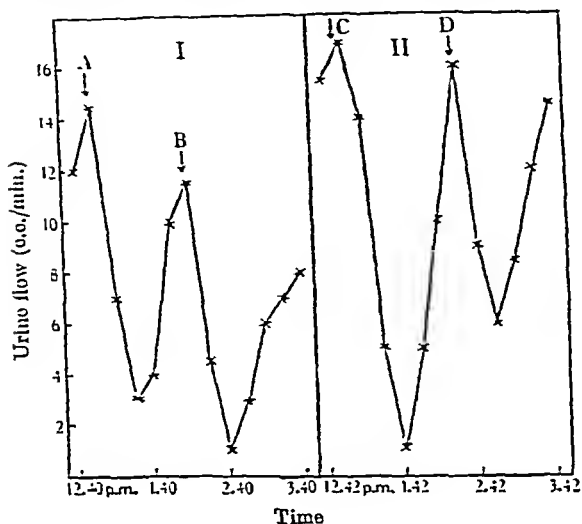


Fig. 3. Estimation of antidiuretic hormone content of a *frog* pituitary gland. Frog no. 12, 20.9 g. ♂. Extract of pituitary gland diluted to 2.0 c.c. I=rabbit no. 77. At 10.25 a.m. and at 12.07 p.m. 5 % of body weight of water by stomach tube. A=0.5 c.c. of frog pituitary extract i.v., B=1.0 mU. pitressin i.v. II=rabbit no. 93. At 10.23 a.m. and at 12.10 p.m. 5 % of body weight of water. C=1.0 c.c. of frog pituitary extract i.v., D=1.0 mU. pitressin i.v. The antidiuretic activity of the frog pituitary extract was equivalent to less than 4 and more than 2 mU. pitressin.

TABLE V. The antidiuretic hormone content of *frog* pituitary glands. Exp. 1, by rat method (see text). In Exps. 2 and 3 the extracts of two frog pituitary glands were pooled

No.	Sex	Weight of animal in g.	Antidiuretic hormone content of pituitary gland in millimits pitressin	
1	—	Four frogs	>10	
2	♂, ♂	22.0, 28.0	> 2	<4
3	♂, ♂	19.7, 22.0	> 2	<6
4	♂, ♂	38.0	> 3	<4
5	♂, ♂	23.0	> 3	<6
6	♂, ♂	54.0	> 3	<6
7	♂, ♂	20.8	> 2	<4
8	♂, ♂	21.1	> 2	<4

D. *Fishes*. (a) *teleosts* (Fig. 4, Table VI), (b) *elasmobranchs* (Table VII).

The figures given for the antidiuretic hormone content of cod, dogfish and skate pituitary glands are likely to be too low as the pituitary glands of those animals were not extracted in a fresh state.

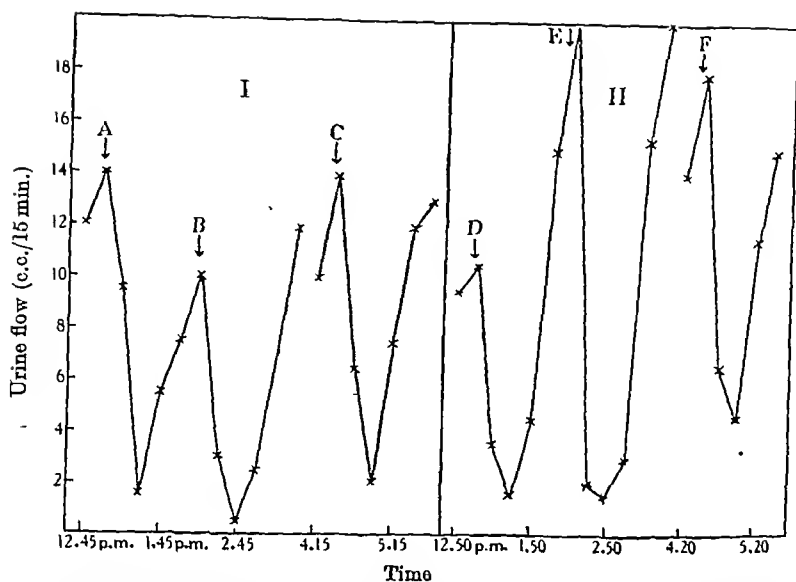


Fig. 4. Estimation of antidiuretic hormone content of a cod pituitary gland. Extract of pituitary gland diluted to 100 c.c. I=rabbit no. 52. At 10.15 a.m., 12.15 p.m. and 3.36 p.m. 5 % of body weight of water by stomach tube. A=1.0 mU. pitressin i.v., B=1.0 c.c. of cod pituitary extract i.v., C=0.34 c.c. of cod pituitary extract i.v. II=rabbit no. 19. At 10.20 a.m., 12.20 p.m. and 3.38 p.m. 5 % of body weight of water. D=1.0 mU. pitressin i.v., E=0.5 c.c. of cod pituitary extract i.v., F=0.34 c.c. of cod pituitary extract i.v. The antidiuretic activity of the cod pituitary extract amounted to clearly less than 300 mU. pitressin (C, F) but to more than 100 mU. pitressin (B). The effect of 0.5 c.c. of the diluted extract (E) was slightly greater than that of 1.0 mU. pitressin (D). Considering this and the pronounced inhibitory effect produced by as little as 0.34 c.c. (C, F) it seems justifiable to assume that the antidiuretic hormone content of this extract was equivalent to slightly over 200 mU. pitressin.

TABLE VI. The antidiuretic hormone content of cod pituitary glands. Cod heads only were obtained. In Exp. 3 the extracts of two pituitary glands were pooled

No.	Antidiuretic hormone content of pituitary gland in milliunits pitressin	
1	>100	<200
2	>200	<300
3	> 50	<150
4	>100	<200

TABLE VII. The antidiuretic hormone content of pituitary glands of *elasmobranch* fishes. In Exps. 1 and 2 the extracts of two pituitary glands were pooled

No.	Weight of animal in g.	Antidiuretic hormone content of pituitary gland in milliunits pitressin	
(a) Dogfish			
1	1222, 1035	>2.5	<5
2	1099, 824	>2.0	<5
(b) Skate			
3	511	>1	
4	610	>1	

Pressor assays of pigeon and frog pituitary gland extracts

Pressor assays were made with extracts of bird and frog pituitary glands. However, no significant difference between the antidiuretic and the pressor potencies of bird pituitary extracts was observed (Fig. 5). The average pressor activity of five pigeon glands equalled that of 69 ± 19 mU. pitressin compared with an antidiuretic activity of about 30 mU. per gland (seven animals). The experiments with frog pituitary glands were inconclusive. The values for the antidiuretic hormone content

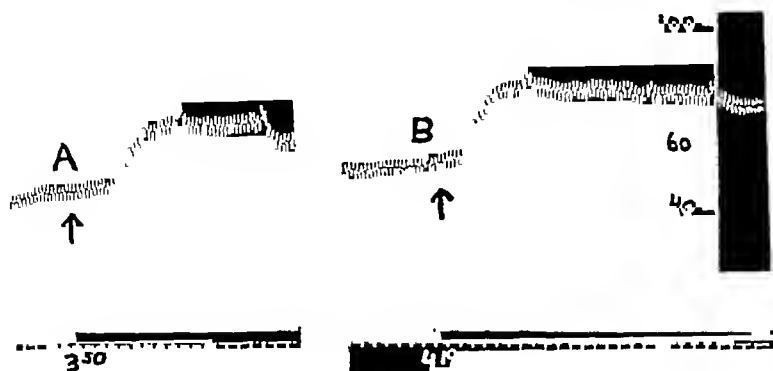


Fig. 5. Pressor activity of *pigeon* pituitary extract. Spinal cat. Extract of pigeon pituitary gland diluted to 3.0 c.c. A = intravenous injection of 1.0 c.c. of pigeon pituitary extract. B = intravenous injection of 20 mU. pitressin. The vasopressor activity of the whole gland equals approximately 60 mU. pitressin.

of cat and rat pituitary glands agree with Simon & Kardos's [1934] estimations of the average pressor activity of pituitary extracts of these animals. Simon & Kardos found an activity of 312 mU. for the cat and 445 mU. for the rat (per 100 g. body weight). The corresponding figures for the antidiuretic hormone content are 400 and 356 mU. respectively.

DISCUSSION

The pars nervosa of the pituitary gland is present throughout the vertebrate series [De Beer, 1926] and a pressor principle has been found in the pars nervosa of all classes of craniates with the exception of the most primitive groups, viz. cyclostomes and elasmobranch fishes. The present series of experiments demonstrates the presence of an antidiuretic principle in birds, amphibians and teleost and elasmobranch

fishes. Cyclostome fishes and reptiles were not investigated. The finding of an antidiuretic activity in elasmobranch glands is in apparent contrast with the absence of a pressor principle, as stated by Herring. However, this can be easily explained by the greater sensitivity of the antidiuretic assay.

Table VIII reveals a striking difference between the amounts of antidiuretic principle present in mammals and that of all other groups of vertebrates. The antidiuretic hormone content (calculated per 100 g. of animal) of any mammalian pituitary gland investigated was at least

TABLE VIII. Antidiuretic activity of vertebrate pituitary glands in terms of milliunits pitressin

Vertebrate class or subclass	Species	No. of animals used	Average weight of animals in g.	Milliunits per gland	Milliunits per 100 g. animal
Mammals	Rat	6	302.0 ± 41.0	$> 920.0 < 1230.0$	$> 305.0 < 407.0$
	Mouse	7	20.8 ± 2.2	$> 27.0 < 48.0$	$> 129.0 < 230.0$
Birds	Pigeon	7	439.0 ± 32.2	$> 21.0 < 41.7$	$> 4.8 < 9.4$
	Drake	3	1865.0 ± 81.3	$> 60.0 < 90.0$	$> 3.2 < 4.4$
Amphibians	Frog (English)	6	29.6 ± 13.6	$> 2.3 < 4.7$	$> 7.8 < 15.8$
Cyclostome fishes	Cod	6	About 6000	$> 116.0 < 216.0$	About 3.0
Elasmobranch fishes	Dogfish	4	1045.0 ± 94.0	$> 2.3 < 4.5$	$> 0.2 < 0.4$
	Skate	2	561.0 ± 62.0	> 1.0	> 0.2

eight times larger than that of any lower class of vertebrates. The question as to the possibility of a correlation between the phylogenetic development of Henle's loop and the post-pituitary hormonal mechanism can thus be answered in the affirmative.

The small amounts of the antidiuretic hormone found in bird hypophyses (Tables III, IV and VIII) suggest a relatively unimportant position of the antidiuretic hormone in the water metabolism of this class of vertebrates. It will be remembered in this connexion that the avian kidney consists of a mixture of the reptilian and mammalian type of nephron and that Henle's loop reaches full development only in the mammal.

Frog pituitary glands contain, per 100 g. body weight, about twice as much of the antidiuretic substance as bird pituitary glands (Table VIII). Is there a significance in the presence of these relatively large amounts in the glands of the more primitive group of vertebrates and in a species in which, according to most authors, an antidiuretic response to injections of post-pituitary extracts cannot be obtained? The possibility must be admitted that the pituitary gland of the frog forms a substance which, though having an antidiuretic effect in the mammal,

has no function in the frog. However, it will be recalled that the anti-diuretic hormone has *two* actions in the mammal: (a) it regulates the excretion of water; (b) it increases the excretion of certain ions. It has recently been shown by Boyd & Whyte [1939] that posterior pituitary extracts exert a similar action on the mineral metabolism of the frog. Boyd & Whyte showed that the active principle thus concerned is contained in the pitressin (antidiuretic-pressor) fraction of commercial post-pituitary extract. It seems significant that it influences the chloride excretion of frogs in very small doses (5 mU. pitressin per 10 g. frog). Considering the amounts of antidiuretic hormone found in the pituitary glands of frogs (about 1.5 mU. per 10 g. frog) and the "unphysiological" method of application of the hormone (injection into a lymph sac), these doses would seem to be within the physiological limit. The question arises, therefore, whether the action of the post-pituitary antidiuretic hormone on the mineral metabolism is not developmentally the older one, the action on renal water reabsorption only appearing with the parallel development of Henle's loop in the mammalian nephron.

This hypothesis does not necessarily mean that the water metabolism of lower vertebrates is not under the hormonal influence of the posterior pituitary gland, but reasons will be given in a later paper to attribute that influence to an active principle not identical with the mammalian antidiuretic hormone.

SUMMARY

1. Quantitative estimations of the antidiuretic activity of pituitary extracts of representatives of the following classes of vertebrates have been made: mammals, birds, amphibians, teleost and elasmobranch fishes. An antidiuretic activity was found in the pituitary glands of all these groups.

2. The pituitary glands of different species of the same class of vertebrates were found to contain roughly the same amount of antidiuretic activity per 100 g. body weight.

3. There is a pronounced difference between the antidiuretic hormone content of mammalian glands and that of all other classes of vertebrates. Mammalian pituitary bodies contained at least eight times as much of the antidiuretic principle (calculated per 100 g. body weight) as the glands of any non-mammalian species (Table VIII).

4. A relation between the phylogenetic development of Henle's loop and the amounts of antidiuretic hormone produced by the posterior

pituitary is suggested, thus correlating the development of an anatomical structure with that of a hormonal mechanism.

The idea of this paper arose during a discussion with Dr C. S. Jang; it is therefore as much his as mine. I wish to thank Prof. J. H. Burn for providing facilities in his department.

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A METHOD OF RECORDING THE RESPIRATION

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MOST of the devices used for recording respiration fail to give a quantitative measure of the air breathed, since the record is affected not only by the depth of breathing, but also by the rate at which each breath is taken. The method described here avoids this fault, and gives a record on which the mean height above the base-line depends upon the total ventilation per minute.

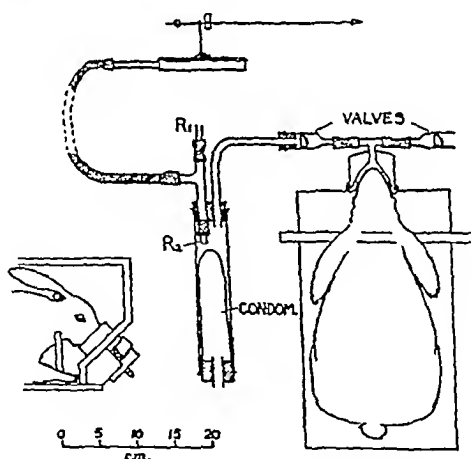


Fig. 1. Apparatus for recording the respiration of a conscious rabbit. The tambour records the fall of pressure in R_1 , and thus the total rate at which the rabbit uses air.

The principle of the method is that all the air breathed by the animal passes, at an approximately steady rate, through a resistance (R_1), and a record is taken of the fall of pressure in this resistance. Fig. 1 shows the apparatus attached to a conscious rabbit by means of a mask. The mask consists of a small tin, on one end of which there is a rubber diaphragm pierced by a hole which fits round the rabbit's nose. A small

T-tube is soldered to the other end of the tin for attachment to valves. In order to diminish the dead space, the tin is filled with plaster of paris, which is moulded to the shape of a rabbit's head and covered with wax. In anaesthetized animals the valves can, of course, be attached to a T-tube which is tied in the trachea.

Valves. It is important that the valves should resist the respiration as little as possible; experiments have been made with various types. The inspiratory valves from service respirators have been found satisfactory both for rabbits and for larger animals. These consist of a rubber ring on to which fits a rubber flap which is thickened in the middle. A piece of rubber dam, fixed at one point so that it covers a hole in a rubber bung, has also been found satisfactory for rabbits. Neither type of valve had any very obvious effect on the respiration.

The tambour. The tambour, which gives a record of the fall of pressure in R_1 , must be very sensitive, to give a record of convenient size without involving enough resistance to obstruct the respiration. The sensitivity depends upon many factors, including the amplification of the movement given by the lever, and the thickness and initial tension of the rubber. The most important factor is the diameter; large tambours are much more sensitive than small ones. The complete calibration curve is S-shaped, and the steepest part of this curve, where the sensitivity is greatest, corresponds to the point where the rubber is flat.

A tambour with a diameter of 11 cm., made from the lid of a tin and covered with medium rubber dam, has given satisfactory results. A round metal disk, weighing 5 g. and with a diameter of about 1 cm., was fixed in the middle of the rubber with sealing-wax, and attached to a light lever with a thread. The weight keeps the thread taut. The lever amplified the movement twenty times. A straight-edge was held across the rubber and the weight on the lever adjusted until the rubber was flat. This tambour gave a displacement of about 30 mm. for 1 mm. of water pressure, when calibrated with Casella's micromanometer.

If the thread is made to pass over a small cylinder which forms the axle of the lever, the movement may be amplified as much as 100 times. The sensitivity is then increased and the curve connecting the height of the record with the pressure applied to the tambour is approximately straight over the range of pressures actually used, but, for reasons discussed later, this is not an advantage.

Fig. 2 shows two tambours working in opposition to record the fall of pressure in a closed circuit. The two metal disks on the rubber are joined by two light, but rigid, wires and the lower disk is attached to

the lever with a thread. The tambours are first adjusted to the correct distance apart, and the weight on the lever is then adjusted until the simultaneous application of the same pressure to both tambours has no effect on the record.

It has been found convenient to attach the mechanism of an electric bell to the stand holding the tambour, so as to set up vibrations which prevent the lever sticking on the drum. This vibrator may have to be adjusted so as to prevent resonance in any part of the mechanism.

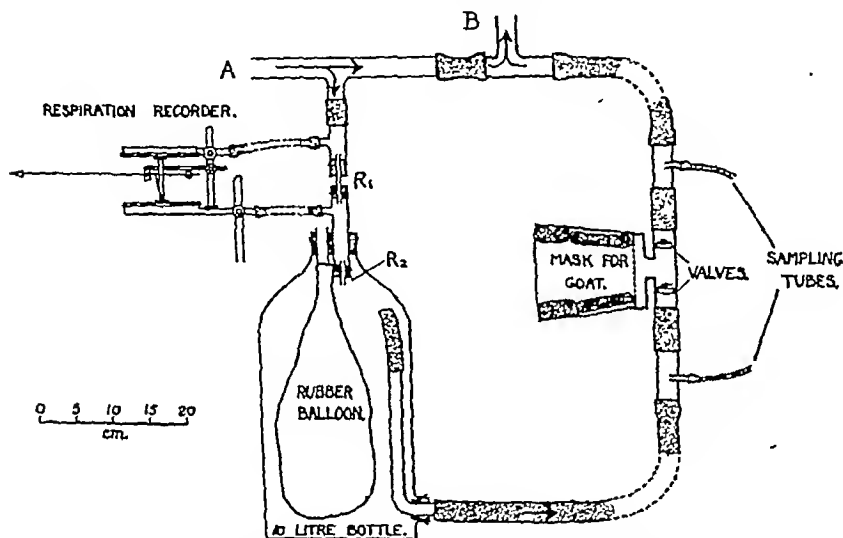


Fig. 2. Apparatus for recording the respiration of a goat, or man, with a closed circuit. A steady stream of air, to which gases can be added at known rates, passes from A to B. Two tambours, working in opposition, record the fall of pressure in R_1 .

The resistance. The air is passed through the resistance, R_1 , before it is breathed, because expired air tends to obstruct the resistance by depositing water in it. Were it not for this fact, it would be better to pass *expired* air through R_1 , so that gases could be administered directly to the animal without passing either through R_1 or through the chamber containing the rubber bag.

The internal diameter of the tube forming R_1 varies from about 2 mm. for rabbits to 10 mm. for horses. The length is about 5 cm.

When the mean velocity of air in a tube is small, the air flows evenly down the tube and the fall of pressure is directly proportional to the rate of flow. When the flow is more rapid the air becomes turbulent, and for very rapid flows the pressure is proportional to the square of the

flow. The relation between the flow (F) and the fall of pressure (P) can be approximately expressed by the equation $P = F^n$. The value of n can conveniently be determined by plotting $\log P$ against $\log F$ and fitting a straight line to the results. For slow flows $n=1$; for rapid flows n is greater. Under the conditions of these experiments n has an intermediate value.

It may be calculated from the formula given by Ower [1933] for the critical flow, at which the air becomes turbulent, is $234d$ c.c. per second, where d cm. is the diameter of the resistance. It would be, in various ways, convenient if the conditions could be chosen so that the rate of flow was below this critical value. In the first place, if the tambour is adjusted so that its calibration curve was straight, the calibration curve of the whole apparatus would then be straight. In the second place it can be calculated that, if the oscillations of the tambour were damped by placing a resistance between it and the rest of the apparatus, intermittent flows would give the same calibration as steady flows.

If the diameter of the tube forming R_1 is large enough to cause turbulence, the sensitivity of the apparatus is seriously decreased. This may be avoided if the length of the tubing forming R_1 is increased, but even with the most sensitive tambour, it was found necessary to use tubes over 1 m. long for rabbits and much longer tubes for larger animals. Since these tubes must be straight to avoid turbulence, the apparatus became unwieldy, and the attempt to avoid turbulence was abandoned.

The conversion of an intermittent flow into a steady flow. When the flow is turbulent and intermittent in R_1 , the average fall of pressure recorded by a damped tambour, depends not only on the total flow of air through R_1 , but also on the wave form of the intermittent change of flow. If the inspiratory valve is connected directly to such a resistance rapid inspirations give higher records than slow inspirations, even when the total air breathed in a given time is the same. It is, therefore, necessary to use some damping device to prevent oscillations of flow from reaching R_1 . In the apparatus shown in Fig. 1 the oscillations are taken up by a thin rubber condom, and prevented from reaching R_1 by a second resistance R_2 , which should be made of slightly smaller tubing than R_1 . Suitable glass tubes for both resistances are found by trial.

Fig. 2 shows a similar device, which has been used for goats and men; the oscillations are taken up by a large rubber balloon such as are used by meteorologists. If the capacity of the chamber which contains the balloon is sufficient there is no need for the balloon. The respiration of a horse has been recorded by using a chamber with a capacity of

10 cu. m. The horse wore a mask with valves, and inspired from the chamber and expired to the open air. The rate of flow of air into the chamber to replace that removed by the horse was recorded by attaching a tambour, between two resistances, to a hole in the chamber.

Calibration. The apparatus must be calibrated on each day that it is used, since the tambour tends to become gradually more sensitive owing to stretching of the rubber. The apparatus shown in Fig. 1 can

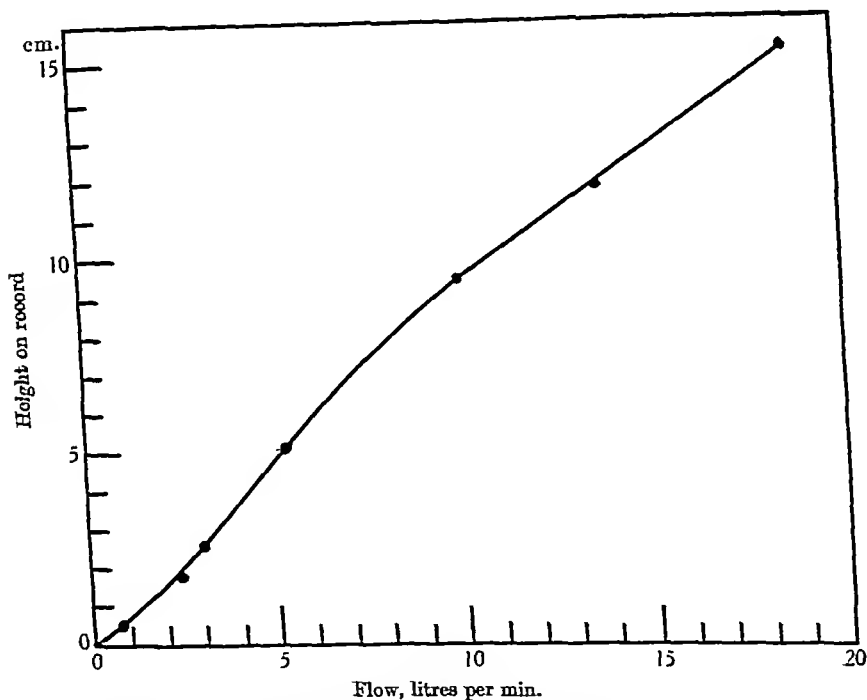


Fig. 3. Calibration curve, using a resistance made from a tube with an internal diameter of 6 mm. and a length of about 5 cm.

be calibrated by allowing water to run from one 10 litre bottle to another, and so sucking air through the resistances at measured rates. With larger rates of flow, the air can be sucked through a gas meter. In order to test the efficacy of the device for damping the oscillations the apparatus shown in Fig. 2 was calibrated both for steady and for intermittent flows. It was found that if the calibration obtained with a steady flow had been applied when the flow was intermittent, the average rate of flow would have been overestimated by about 10%. This error is small compared with the variations between individual animals and was not considered

important, since the main use of this type of apparatus is to record changes of respiration. It could probably have been diminished increasing the resistance at R_2 .

If the flow is plotted horizontally and the fall of pressure vertical the curve is concave upwards when the flow is turbulent, and sensitivity increases as the flow increases, so that the writing point liable to go beyond the edge of the smoked paper. With the tambour

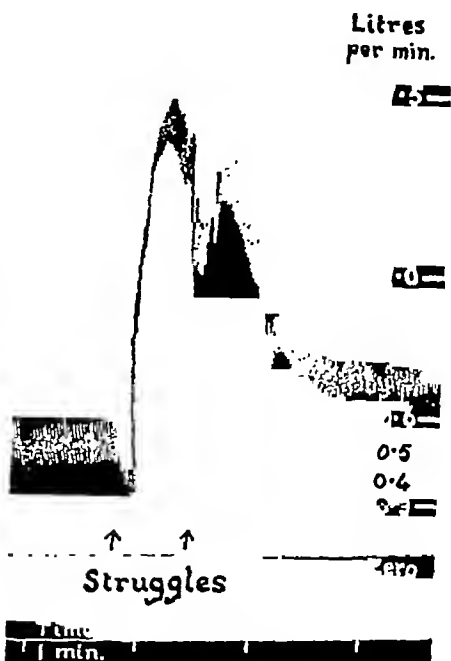


Fig. 4. Record of the respiration of a rabbit.

described above, this source of trouble is avoided since the calibration curve of the tambour itself is not linear over the whole range, and the tambour becomes insensitive when the record would tend to rise too rapidly.

These facts are illustrated by the shape of the calibration curve shown in Fig. 3, for which R_1 was a tube with an internal diameter of 6 mm. The curves obtained when tubes with internal diameters of 7 and 9 mm. were used, were the same shape provided that the horizontal scales were multiplied by factors of 0.54 and 0.25 respectively. These

results are consistent with the finding that the resistance is proportional to the fourth power of the diameter of the tube [Ower, 1933].

When plotted on double logarithmic paper, the calibration curves become practically straight over the important part of their course, and the curves for different resistances are parallel.

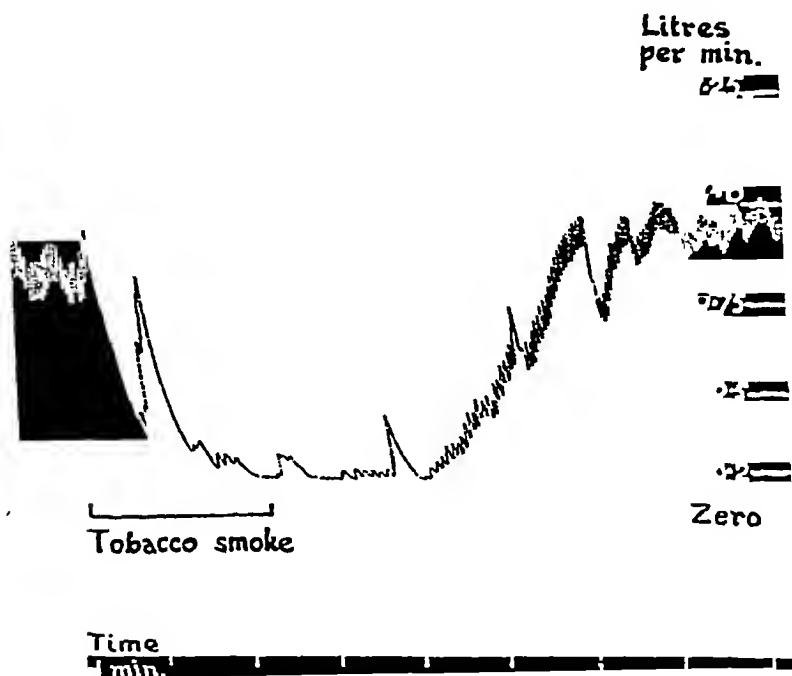


Fig. 5. Effect of tobacco smoke on the respiration of a rabbit.

RESULTS

Fig. 4 shows the type of tracing obtained with a conscious rabbit, which at first sat very still and then tried to free itself from the mask. During the actual struggles there was a fall in the total ventilation, but this was followed by a large rise. Both the total ventilation and the rate of respiration were increased.

Fig. 5 shows the effect of holding a lighted cigarette near the inlet. The rabbit reduced its ventilation to about 20% of its former value for several minutes ("Kratschmer's reflex").

SUMMARY

A simple method of recording the volume of air respired per unit time is described. The method can be applied either to conscious animals or to anaesthetized animals.

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THE SECRETION OF URINE BY NEWBORN INFANTS

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(Received 16 August 1940)

INTEREST has been aroused from time to time in the period of foetal life at which the human kidney first begins to secrete urine. Very little work has been done on the matter, but it has been shown that some functions are in evidence at least as early as the second or third month [Cameron & Chambers, 1938; Hewer, 1924]. Analysis of liquor amnii at serial stages of foetal development has indicated that long before birth some urea and uric acid are being excreted by the kidney [Guthmann & May, 1930]. According to Tausch [1936], who reviewed the literature on foetal kidney function and himself catheterized a great number of infants immediately after delivery, the urine of the last few hours or days of foetal life is generally acid, and has a lower specific gravity than that found in adults. It contains creatinine and sometimes albumen, and the mean percentage of non-protein nitrogen amounted in that series to 0.155%. As an index of functional capacity, any results of this nature are of qualitative interest rather than of quantitative importance, because until birth the placenta probably carries out most of the duties which will later be taken over by the kidney. At birth the kidney must be capable of assuming enough of its adult functions to enable life to proceed, but there is extraordinarily little knowledge about the physiology of the kidney at this important age, or indeed for the next few critical years of the infant's life. This is the more remarkable because the gross lobulation of the kidney at birth suggests that it is still in an active stage of anatomical, and why not therefore of functional development?

Taylor, Drury & Addis [1923] found that in rabbits the weight of the kidney and its capacity to excrete urea varied with the surface area of the body. Mackay & Mackay [1927-8] concluded that in rats "the

kidney weight has practically the same relation to body surface as ages". Neither group of workers, however, tested really young animals. Mackay's youngest rats were about 35 days old and weighed about 6 g. Taylor *et al.*'s lightest rabbits weighed 453 g. and were probably six weeks old. Following their work on adults, McIntosh, Möller & Van Slyke [1928] studied the urea clearances of eight normal children, the youngest aged $2\frac{3}{4}$ years. They found that when the volumes of urine were "corrected" to a standard surface area, the urea clearances of children were of the same order as those of adults. Holten [1930] extended these investigations to ninety children, of whom, however, only two were under 2 years of age. He arranged his experiments to get high urine volumes and found the clearances of exogenous creatinine to be roughly proportional to surface area from 9 months to 17 years of age. He also found that the concentrating power of the kidney was as great at 4 years as it was in later childhood and adult life. At a comparatively early age, therefore, many of the adult functions have become fully established. The present investigation, however, which has been limited to children below 1 year of age, has shown that for some time after birth the kidney does not function as it does in adult life, and it is submitted that this fact is one of importance, not only to physiology, but also to pathology and medicine.

MATERIALS AND METHODS

Normal full-term male infants were the subjects of the main investigation. Their ages ranged from 7 to 14 days (Table I), but only two were over 9 days old. After one passage of urine had been accurately timed, a hard glass test tube was fitted over the penis and attached with a small amount of strapping. Into such tubes the infants were allowed to void their urine spontaneously. Three consecutive specimens were collected. Each was removed as soon as it was passed. Children of this age scarcely require restrainers, but in some instances the legs were loosely attached to the sides of the cot by bandages over pads round the heels. Few specimens were incomplete once the staff had had the necessary practice and it was even possible to lift the children and put them to the breast without disturbing the collections. Hence the tests did not interfere with the normal feeding regime.

Blood was collected from a prick in the heel at some time during the test. Whole blood was used for the urea determinations, serum for the metallic radicles, and heparin plasma, taken and separated under paraffin, for the chlorides. A supplementary investigation was carried

out on one female and two male infants with meningoceles. These children were aged 6 to 13 days like the others, but they can hardly be regarded as strictly normal. One had hydrocephalus, another had some pyrexia with ulceration over the meningocele, and their results therefore will be presented separately. 4-8 g. of inulin were administered intravenously to these babies before the function tests were to be made. Samples of blood were withdrawn at suitable intervals through the anterior fontanelle as the concentration of inulin fell in the plasma. A curve for the plasma inulin was then drawn covering the experimental periods. In calculating clearances, values at the mid point of each period were read off from the curve. The urines in these tests were collected by catheter and diluted while still warm to maintain the inulin in solution. It was not always easy, however, to empty the bladder satisfactorily, nor again to prevent leakage of urine round the catheter as the bladder filled. A number of specimens had to be discarded in consequence, but all the same the observations made on these children have been of considerable value.

Urea was determined by Lee & Widdowson's [1937] method, inulin by Herbert's [1938] method for fructose. The readings in both were made by means of the Evelyn photoelectric colorimeter. Chlorides in plasma were determined by Claudius's ultra-micro application of the open Carius method; in urine by Van Slyke & Sendroy's method [Peters & Van Slyke, 1932]. Sodium was determined by the pyroantimonate technique [Peters & Van Slyke, 1932]. Potassium was determined in serum by an adaptation of the iodometric micro-method described by Dénes [1939]; and in urine by the cobaltinitrite method [Peters & Van Slyke, 1932].

RESULTS

The serum chemistry

A study of the serum chemistry is an important part of any investigation of kidney function, and Table I shows the results which have been obtained. It will be seen that the figures differ in certain respects from the accepted normals for adults, and from the findings in the eight normal adults who have been examined by the same methods as controls. The blood ureas were inclined to be low, sometimes very low. Only two of the babies had blood ureas as high as 30 mg./100 c.c. and these differed somewhat from the others, for they were about a week older and they were not being fed on breast milk, but on a $\frac{1}{2}$ cream Cow and Gate preparation. This meant that they were having about 8.4 g. of

TABLE I. Serum chemistry of infants, 7-14 days old, and of adults

Subject	Age in days	Infants			
		Urea mg./100 c.c.	Na mg./100 c.c.	Cl mg./100 c.c.	K mg./100 c.c.
1	8	15.0	346	385	31.5
2	8	14.0	—	398	—
3	14	30.0	338	366	35.0
4	13	33.0	353	410	27.8
5	7	10.0	336	355	31.7
6	7	15.5	333	355	34.0
7	7	12.6	—	415	—
8	8	26.4	—	413	—
9	9	13.0	—	372	—
10	8	13.0	340	398	28.4
11	8	9.0	309	364	35.2
12	8	22.0	297	354	40.3
13	7	10.0	328	364	18.5
14	7	14.0	372	372	35.5
15	7	18.0	291	386	—
16	7	11.5	—	373	32.0
17	8	14.0	—	373	—
18	9	11.0	336	398	17.0
Averages		16.4	332	381	30.6
S.D.		6.3	26.2	19.6	6.5
Subject	Age in years	Adults			
		Urea mg./100 c.c.	Na mg./100 c.c.	Cl mg./100 c.c.	K mg./100 c.c.
1	28	24	348	375	19.0
2	25	28	336	372	22.4
3	25	18	339	372	21.4
4	30	35	323	357	19.1
5	28	22	327	375	25.5
6	30	28	334	367	21.2
7	33	25	318	365	23.2
8	41	36	334	374	22.8
Averages		27	332	370	21.9
S.D.		5.7	8.9	5.9	2.1

protein per day instead of 4.5 g. The additional protein in their food may have been the sole cause of their higher blood ureas.

The sodium and chloride in the serum of the newborn infants were often within the adult range, but some of the chloride values were distinctly high. Two of the serum potassiums were lower than any of the adults; but the majority were very high, and frankly abnormal by adult standards. So far as potassium is concerned these results must be regarded as *confirming* those of Kotikoff [1933], who found high serum values for this element in the first month of life. Kotikoff suggested that the high serum potassiums were the result of the rapid post-natal destruction of red blood corpuscles, with liberation of their contents. A preliminary examination of cord blood, however, by Bassadone [1940] has indicated that the serum potassium may be high at birth. Kotikoff's figures for sodium are so much lower than the present ones and the

accepted values for adults, that it is probably better to neglect them. The differences between the infantile and adult averages for urea, chloride and potassium are statistically significant, and it will be noted from the comparative magnitudes of the standard deviations how much more variable each constituent was from one infant to another than from one adult to another.

The serum chemistry of the children with meningoceles need not be given in detail. The infant with pyrexia had a blood urea of 61 mg./100 c.c. and none of the three had serum potassiums over 24 mg./100 c.c., but the other findings were typical.

The urea clearances

Before considering the urea clearances in infancy it is important to grasp the principles which have been found to hold for the excretion of this compound in adult life. When the volume of urine (V) exceeds about 2 c.c./min. it has been found that the amount of urea excreted per minute is independent of the urine volume. The urea clearance is constant and averages 72 c.c./min. in a man with a surface area of 1.73 sq. m. ($UV/B=K$, where K is a constant and U and B are the percentages of urea in urine and blood respectively). As the urine volumes fall from about 2 c.c./min., which is often referred to as the augmentation limit, to about 0.35 c.c./min., the concentration of urea in the urine rises but the urea clearance falls, and at a urine volume of 1 c.c./min. averages 54 c.c./min. Over this range of minute volumes $U\sqrt{V}/B=K'$. At or about a urine volume of 0.35 c.c./min. the urea clearances average 32 c.c./min. and a decrease of urine volumes below this figure does not lead to a higher concentration of urea in the urine, so that the urea clearances vary directly with the minute volume and $U/B=K''$. It is further agreed that the glomerular filtration rate does not begin to fall till the urine volumes have fallen well below 2 c.c./min., but it has been suggested on the basis of the endogenous creatinine clearances that at minute volumes below 0.35 c.c. the glomerular filtration rate also varies directly with the minute volume [Chesley, 1938; Steinitz & Türkand, 1940].

Fig. 1 shows the urea clearances (UV/B) of the eighteen children plotted against their minute volumes (V). Each point represents one clearance and lines connect the separate clearances of each child. It will be noted that: (1) The minute volumes varied from 0.022 to 0.38 c.c. (2) The magnitudes of the clearances and of the minute volumes varied considerably from one child to another. (3) The clearances of most

babies increased steadily with the minute volume. Nos. 3, 4, 5, 7, 11, 14, 16 and 18 showed this particularly well. Minor irregularities were provided by Nos. 6, 8 and 15, and response to an increase of minute volume was inconsistent in Nos. 13 and 17. No. 9's clearances fell as the minute volume rose. Neglecting these exceptions for the moment, the general relationship of the infants' clearances to their minute volumes

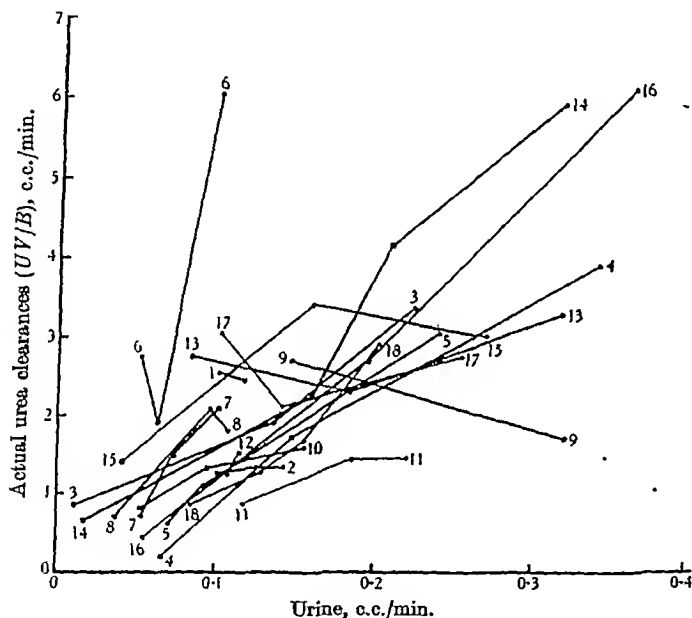


Fig. 1. Individual urea clearances of newborn infants.

suggests that the latter are all well *below* the so-called "augmentation" limit, above which in adults the clearances become independent of the minute volumes. (4) The U/B ratios, which are not shown directly in Fig. 1 but which may be obtained by dividing the value of any clearance by the corresponding minute volume, are of the order of 15. There are exceptionally high ones, e.g. No. 6, but the general level is very low and relatively constant. Such ratios would only have been obtained in adults with minute volumes well *above* the augmentation limit of 2 c.c./min. It is apparent, therefore, that there are certain differences between the U/B -minute volume relationships of adults and infants.

Comparison with adults

There is no a priori reason why an infant's ability to concentrate urea should be less than that of an adult. It is obvious, however, that the total amount of urea excreted per day and the total volume of urine must be much less. It is therefore essential to apply some "correction factor" if infant and adult clearances are to be compared. From McIntosh *et al.* [1928] and from Holten's [1931] work it was expected that these infants' clearances would fall into line with those of adults when the volumes had been "corrected" for surface area. The following consideration, moreover, shows that this was a reasonable correction to make so far as the excretion of water was concerned. Adults, living in a temperate climate and passing about 1500 c.c. of urine per day, are generally thought to excrete rather more than 50% of their total water intake by the kidney [Harrison, 1937; Du Bois, 1936]. Their urine volumes are of the order of 0.6 c.c./sq. m./min. Only a few of these newborn infants were as yet being given the $2\frac{1}{2}$ oz. of fluid per lb. of body weight per day which is recommended for young children. They were getting between 252 and 475 c.c./day. Their urine volumes while under observation were of the order of 0.1-0.15 c.c./min. (Fig. 1), i.e. about 180 c.c./day, or 0.6 c.c./sq. m./min., which agrees well with the adult figure.

In order to simplify the comparisons with adults the infants' clearances were averaged. At various minute volumes (see Fig. 1) vertical lines were drawn cutting the complex of lines joining the separate determinations. The values of the clearances at these minute volumes were read off from the points of intersection and the mean found. Baby No. 6 was omitted from the average as being exceptional. The results are shown in Fig. 2 compared with the accepted values for normal adults. It may be mentioned that the adults used as controls in this investigation gave clearances at all minute volumes which were within normal limits and near the adult line. It is evident that the infant clearances are very much below the adult level when surface area is taken as the basis of comparison, and that, therefore, Holten's [1931] and McIntosh *et al.*'s [1928] generalization does not apply to infants. It is interesting to note in passing that the line relating these averaged infant clearances to the minute volume is almost straight, and if prolonged to the left would pass near the origin. This is reminiscent of the relationship at very low minute volumes in adults when the U/B ratio has become constant. The slope of this line gives the average U/B ratio. Fig. 2, therefore, also

shows very well that the infantile are lower than the adult clearances, because at comparable minute volumes their U/B ratios are much smaller.

Surface area was originally chosen as the basis on which to compare clearances on the grounds that, both in young and old, kidney weight and basal metabolic rate were proportional to surface area. These

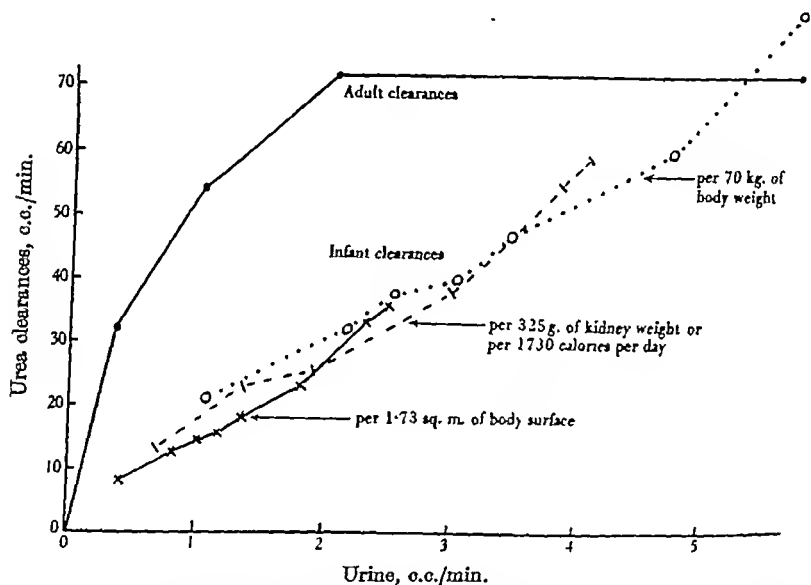


Fig. 2. A comparison of adult and infantile urea clearances on the basis of surface area, kidney weight, total basal energy requirements, and body weight. To enable comparisons on four different bases to be made on a single chart, the average UV/B has been plotted against V for a "theoretical" man with a surface area of 1.73 sq. m., total basal requirements of 1730 calories/day, whose kidneys weighed 325 g. and whose body weight was 70 kg. The infant minute volumes, and hence clearances, have been "corrected" to the adult basis by multiplying them by the adult/infantile ratio for surface area, body weight, etc.

relationships, however, do not hold for the newborn [Vierordt, 1888; Mühlmann, 1927; Bailey & Murlin, 1913-14, 1915; Benedict & Talbot, 1914; Benedict, 1919; Benedict & Talbot, 1921; Talbot & Sisson, 1921-2; Talbot, Sisson, Moriarty & Dalrymple, 1922; Levine & Marples, 1931; Du Bois, 1936]. The approximate relationships have been taken from the literature and are shown in Table II. It is interesting to note the correlation between kidney weight and total energy requirements for basal purposes. A comparison of the infant and adult clearances on the basis of kidney weight, body weight and total basal metabolic require-

ments are also shown in Fig. 2. It is evident that on any reasonable basis of comparison the infants' clearances are much lower than the adults'. The infant clearances reach the adult level on the basis of body weight, but only at a minute volume which would correspond to one of more than 5.3 c.c./min. in a normal adult. The considerations outlined above make it impossible to suppose that the infants were excreting volumes of urine of a corresponding magnitude.

TABLE II. A comparison of the weights, areas and metabolism of newborn infants and adults

	Surface area sq. m.	Basal metabolic rate per sq. m. per day	Total basal requirements per day	Kidney weight g.	Body weight kg.
Adults	1.73	1000	1730	325	70
Infants	0.21	650	137	24	3.27
Ratio	8.25	1.54	12.6	13.5	21.4

The mineral clearances

Very much less is known about mineral than about urea, inulin or creatinine clearances, and no adult standards have been laid down. The clearance of a true non-threshold substance such as inulin or urea should only be affected by the glomerular filtration rate and perhaps by the minute volume, but the clearance of a threshold body such as glucose or chloride depends not only upon the glomerular filtration rate and the minute volume, but also upon the concentration in the serum and the reabsorptive activity of the tubules. This last is partly controlled by the suprarenal cortex—at any rate so far as sodium, chloride and potassium are concerned—and it is not possible to make any measurement of the extent of this. It is, however, possible to measure the serum concentration and, other things being equal, one would expect the clearance of a threshold substance to rise from 0 to half the glomerular filtration rate as the serum concentration rose from the threshold to twice the threshold value. In short, a small increase in the normal serum level of any of these electrolytes should produce a large rise in the clearance value. A comparison of the serum chemistry of the adults and the infants (Table I) shows that on average the infants' serum sodium was as high as the adults' and the chloride and potassium significantly higher. Other things, therefore, such as tubular reabsorption and minute volumes, being equal, the infant clearances of sodium should have been as high, and of chloride and potassium higher, than those of adults. In order to make the comparison the surface area basis was adopted, since this is probably the correct basis for water excretion at all ages

and for urea and creatinine in older children. The sodium and chloride clearances are given in Fig. 3, and the potassium clearances in Fig. 4. As in Fig. 1, each point represents one clearance and lines connect the separate clearances of each child. The sodium and chloride clearances vary like the urea clearances with the minute volume, but far from being higher, the infant clearances quite evidently tend to be lower than the adult clearances. There is practically no overlap where sodium and chloride are concerned, but two of the adults gave quite low potassium

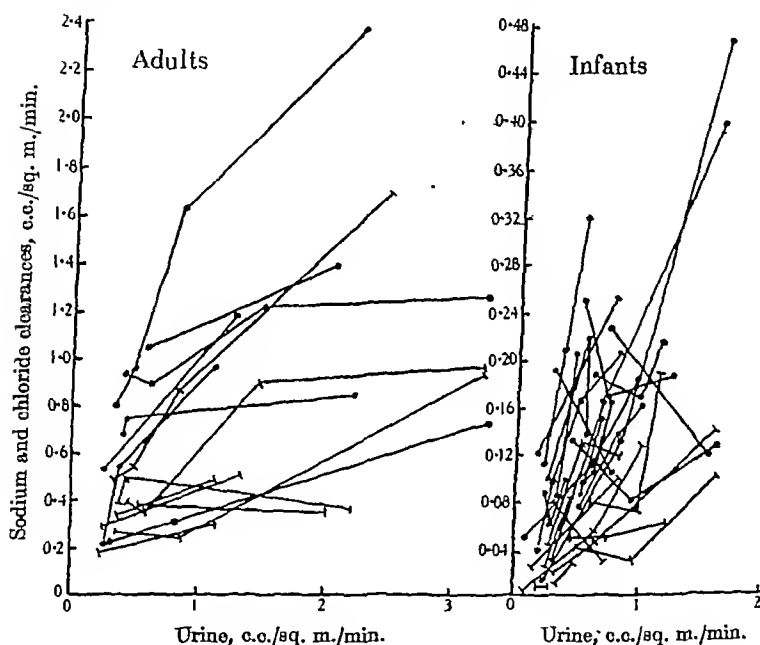


Fig. 3. A comparison of the sodium and chloride clearances of adults and newborn infants. Chloride clearances $\bullet-\bullet$, sodium clearances $|-|$. Note. The infant scale is $\frac{1}{3}$ that of the adult scale.

clearances, which were exceeded by two or three infants, and one of the infants produced a clearance greater than most of the adults and enormously greater than those of any of the other infants. This baby had a high serum potassium (27.8 mg./100 c.c.), but others had higher. A gross change in the concentration of sodium, potassium or chloride in the serum might have been expected to alter the clearance in the same direction, but it did not seem justifiable to give large doses of these minerals to babies of this age for purely experimental purposes, so it was impossible to prove this point in individual children. A study of the

serum levels in different children, moreover, with their corresponding clearances has been of no help, for individual idiosyncrasies, of which we have no certain knowledge, far outweigh the influence of the absolute serum levels on the clearances. This is demonstrated in Table III, in which the potassium and chloride clearances at different levels of urine

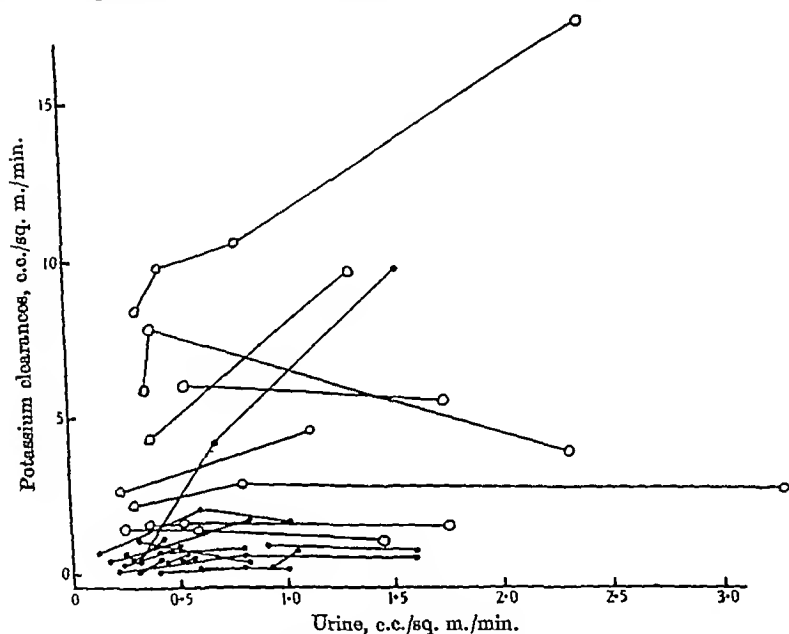


Fig. 4. A comparison of the potassium clearances of adults and newborn infants.
Adults o—o, infants •—•.

TABLE III. Serum levels of potassium and chloride in different children set against their actual mineral clearances at comparable rates of urine flow

Serum K mg./100 c.c. (min. vols. 0.06-0.10 c.c.)	K clearance c.c./min.	Serum K mg./100 c.c. (min. vols. 0.1-0.15 c.c.)	K clearance c.c./min.	Plasma Cl mg./100 c.c. (min. vols. below 0.10 c.c.)	Cl clearance c.c./min.
17.0	0.098	27.8	0.920	355	0.013
18.5	0.146	31.5	0.110	355	0.023
27.8	0.106	35.0	0.456	355	0.045
31.7	0.013	35.2	0.056	355	0.020
34.0	0.265	40.3	0.148	364	0.026
34.0	0.087	40.3	0.094	366	0.011
35.5	0.249	(min. vols. 0.15-0.20 c.c.)		372	0.039
		17.0	0.406	373	0.004
		18.5	0.167	386	0.025
		28.4	0.159	398	0.018
		35.2	0.053	398	0.033
		35.5	0.094	410	0.008
				413	0.007
				415	0.025
				415	0.040

flow have been set against the plasma values. It would be very interesting to know what the personal factors are which override the expected relationships between plasma level and clearance.

The mineral clearances of the children with meningoceles require no comment except to point out that two of them on occasion gave rather high sodium and chloride clearances. Baby B had a chloride clearance of 0.51 c.c./sq. m./min. during his intense diuresis (see Table IV) and

TABLE IV. The relationships of minute volumes, inulin and urea clearances

Name	Min. vol. c.c.	Inulin clearance c.c./min.	Urea clearance c.c./min.	Urea Inulin clearance ratio
B, 1st day	0.040	1.12	0.283	0.253
	0.203	5.50	1.870	0.340
	0.387	6.10	1.980	0.324
	0.452	7.50	2.580	0.346
	0.697	8.10	3.720	0.460
	0.770	9.80	3.400	0.352
B, 2nd day	0.030	5.02	1.720	0.343
	0.045	5.40	1.870	0.346
	0.055	5.12	1.420	0.277
	0.063	5.95	1.870	0.314
	0.193	6.60	2.280	0.346
S	0.117	3.67	1.490	0.402
	0.193	5.92	2.100	0.360
H	0.109	5.40	1.730	0.320
	0.134	8.50	3.900	0.460
Very large		—	—	0.710

baby H had one chloride clearance of 0.75 and one sodium clearance of 0.38 c.c./sq. m./min. respectively. These must be regarded as exceptional figures for infants and may be connected with the fact that the inulin given intravenously had been dissolved in 40–60 c.c. of normal saline.

The osmotic pressure of the urine

One of the characteristic properties of the mammalian kidney is its power to secrete a urine with a higher or lower osmotic pressure than that of the blood. This power of the kidney to produce a hyper- or hypotonic urine has been made the basis of a number of function tests, for as the kidney becomes progressively damaged by nephritis the osmotic pressure of the urine which it secretes tends to become more and more fixed and nearer and nearer to that of the blood [Fishberg, 1930]. Functional disorganization of a different type may occur in certain diseases. The kidney may then produce small volumes of hypotonic urine from a plasma from which it would normally produce a highly concentrated urine [McCance & Widdowson, 1939; Allott, 1939]. Since the

osmotic pressure of the blood is largely made up by the salts of sodium and that of the urine by chlorides and urea, an indication of the relative osmotic pressures of the urine and blood may be obtained from the ratio

$$\frac{(\text{m. eq. of Cl in the urine} \times 2) + \text{m. eq. of urea}}{(\text{m. eq. of Na in the serum} \times 2) + \text{m. eq. of urea}}$$

This relationship has been worked out for each of these specimens of infants' urine with the refinement that m. eq. of Na+K in the urine have been substituted for m. eq. of Cl if the former were higher than the

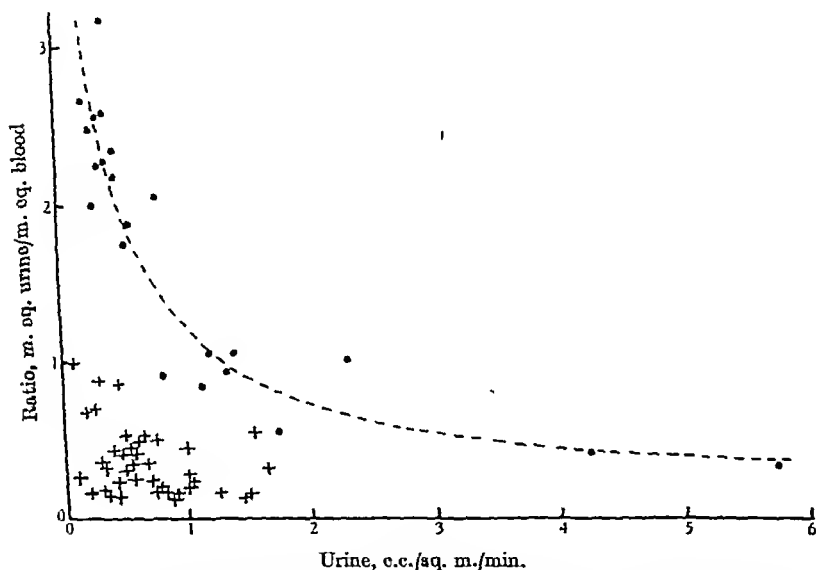


Fig. 5. A comparison of the urine/blood osmotic pressure ratios produced by adults and by newborn infants at comparable urine volumes. Adults •, infants +.

latter, and that m. eq. of K have been included in the computation of the serum. The result has been to show that infants have very low *U/B* ratios. In this series of forty determinations only one was found to be as high as 1 and thirty were below 0.5. To set these ratios against those of adults it is again necessary to place the minute volumes on a comparable basis.

Surface area has been chosen for the reasons already given and the results are shown in Fig. 5. One must conclude that the kidneys of these infants were functioning very differently from those of the adults, for they were consistently producing hypotonic urines from plasmas (Table I) from which adults would certainly have produced hypertonic urines at

comparable minute volumes. The findings in the supplementary investigation have corroborated these conclusions. Babies H and S (Table) both of whom had high chloride clearances, each produced a specimen of urine with a U/B ratio slightly over 1, but baby not do so even on the day on which his fluids were restricted by omission of two feeds before the tests were carried out. This is perhaps the best available evidence that some newborn babies may be unable to produce a hypertonic urine.

DISCUSSION

At the outset of this discussion it is well to emphasize that the present results are not in any sense a contradiction of those of McIntosh [1928] or of Holten [1931]. The children studied were not the same

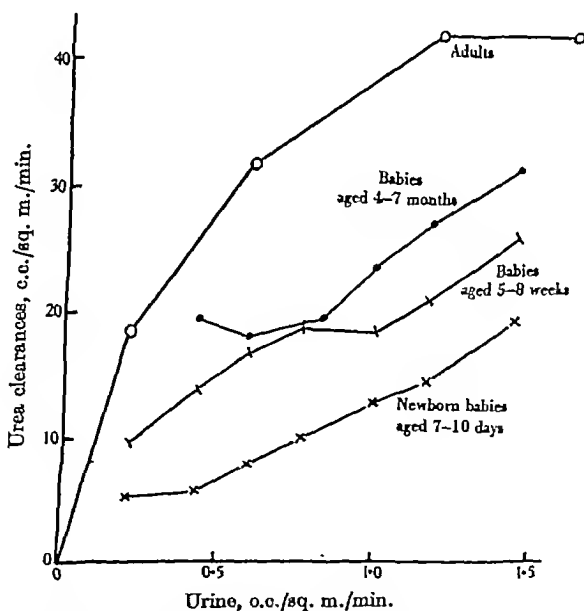


Fig. 6. A comparison of the average urea clearances of adults and of infants at three different ages.

There is good reason, moreover, to think that these previous results would have been confirmed if older children had been investigated. The evidence for this statement comes from a study of four children aged 5 to 8 weeks and six children aged 4 to 7 months. When the urine volumes of these older children were "corrected" for surface area, the urea clearances fell between those of the newborn infants and those

accepted adult levels. The averaged results of the four groups are shown in Fig. 6, from which it appears that the clearances rise rapidly in the first year of life. Since Holten [1931] worked with high minute volumes, it is not surprising that his one subject aged 9 months gave a result within the normal adult limits.

The present observations have raised a number of questions, and by trying to answer them the results may be discussed:

(1) What is the relationship between the low urea clearances of infancy and the glomerular filtration rates?

If the inulin clearance may be taken as a measure of the glomerular filtration rate, the subsidiary investigation on the three infants with meningoceles provides a tentative answer to this question. Their inulin clearances were of the order of 30 c.c./sq. m./min. Adult clearances should be about 70 c.c./sq. m./min. [Smith, 1937]. It will be seen moreover, from Table IV that on the whole the inulin clearances varied with the minute volumes, and that, speaking generally, the ratio of the urea clearance to the inulin clearance was little affected by the minute volume. These ratios were rather small by adult standards. Hence we may conclude that in infancy the glomerular filtration rate is relatively low, but varies quite extensively with the minute volumes, and that about two-thirds of the urea filtered off in the glomeruli is reabsorbed by the tubules. In adults, on the contrary, the glomerular filtration rate is relatively high and constant until the minute volumes become very small [Smith, 1937; Chesley, 1938]. This is certainly true of dogs. It is to be noted, however, that in rabbits the glomerular filtration rate has been found to vary with the minute volumes, even when these are large [Kaplan & Smith, 1935; Wilkinson & McCance, 1940].

(2) Why are all the infantile clearances so low that the urine is almost always hypotonic?

(a) If the glomerular filtration rates are low the urea clearances must be low, and the clearances of threshold bodies such as chlorides will also be low if the capacity of the tubules to reabsorb them is relatively high and well developed. This combination of subnormal glomerular filtration and normal tubular reabsorption was the explanation put forward by McCance & Widdowson [1939] to explain how it came about that high plasma chlorides and glucose could be accompanied by chloride and sugar-free urines. A knowledge of the glucose threshold in infancy would be of help in this connexion. Anatomically the suprarenal cortex is hypertrophied at birth. If this indicates great functional activity, then excessive cortical secretion might be invoked to explain the low sodium

clearances; not, however, the low potassium clearances, which one has expected to be high if the cortex were overactive.

(b) The kidney is an organ which at all ages is capable of hypertrophy in response to additional work. Till birth the kidneys probably have half their work done for them by the placenta, and may take them some little time after birth to hypertrophy in response to the additional demands. It is thought that this would explain the finding of low urea clearances and isotonic, but not hypertonic, urines.

(c) At birth certain parts of the human kidney are still undeveloped. According to Peter [1927] the glomeruli are all formed, but the ones near the capsule are still very primitive, and the loops of Henle very short. The most central nephrons are in a much more advanced stage of development, but even they have many primitive features. If the loops of Henle were functionally defective at birth the kidney might be unable to reabsorb enough water to produce a concentrated urine, and could then be described as being still in the stage of functional development characteristic of the fish and amphibia. Unfortunately, conclusions about function based upon measurements of the loops of Henle are complicated by the fact that even in the adult the loops are of very different lengths [Peter, 1909].

(3) Seeing that the infantile kidney is such a relatively inefficient organ, how comes it that it is able to do its job so satisfactorily?

There seem to be two answers to this question:

(a) Human milk is such a perfectly constituted food for the newborn infant, and the rate at which new body tissues are being laid down is so great, that for some time after birth the kidney has relatively little to do.

(b) The infantile kidney is only capable of maintaining a normal serum when everything is going well. Ribadeau-Dumasse & Seguiet & Mignon [1937], for instance, showed that a minor change in diet may produce surprising changes in the blood urea, and in a series of babies of 2-7 months, recently studied, the blood urea was found to be higher in those which were being artificially fed, and then on a greater protein intake, than in those which were being breast-fed. A study of infants' blood chemistry in diseases likely to throw even a mild strain upon their kidneys has revealed surprising departures from the normal. It is hoped to expand this aspect of the matter in a future publication.

(4) Have the present discoveries any therapeutic significance?

They certainly have, for:

(a) Since the clearances of inulin, urea, sodium and chloride have all been found to be very low in infancy but to increase with the minute volume, it is clearly important to keep the fluid intake at such a level that the output by the kidney is maintained. Oliguria in fact is synonymous with renal insufficiency.

(b) The results show how necessary it is to control the amount of protein in the diet, especially when the urine output is diminished during periods of pyrexia, or increased fluid loss by the gastro-intestinal tract.

(c) They emphasize particularly how carefully saline should be given to infants. Normal saline rightly enjoys a great reputation as a therapeutic agent, but its administration to adults requires care [Jones & Morgan, 1938], and, if the infantile kidney finds it difficult or impossible to excrete a hypertonic urine, then clearly normal saline ought to be placed on the list of dangerous drugs, only to be used after a careful consideration of dosage.

SUMMARY

An investigation of the kidney function of newborn infants has shown that:

1. Their minute volumes varied from 0.022 to 0.38 c.c. This corresponds very well with the adult range when both are expressed on a surface area basis.

2. Their urea clearances varied as a rule with the minute volume, but were always very low by adult standards whether compared on a basis of surface area, kidney weight, or body weight.

3. Their sodium and chloride clearances were very low by adult standards even when the plasma values were abnormally high.

4. Their potassium clearances were low although the serum potassiums tended to be high and were sometimes twice the normal adult level.

5. Their urines were always hypotonic and generally extremely so.

A consideration of 2, 3, 4 and 5 leads to the conclusion that the kidney of the very young infant is a relatively ineffective organ, the causes and significance of which have been discussed.

6. A supplementary investigation on three newborn infants with meningoceles has shown that their inulin clearances were of the order of 30 c.c./sq. m./min. at normal urine flows. These clearances varied with the minute volumes and were $2\frac{1}{2}$ –3 times the urea clearances. The ratio of the urea/inulin clearances did not alter consistently with the minute volume. Hence, it is concluded that in infancy the glomerular filtration

clearances; not, however, the low potassium clearances, which one would have expected to be high if the cortex were overactive.

(b) The kidney is an organ which at all ages is capable of great hypertrophy in response to additional work. Till birth the kidneys probably have half their work done for them by the placenta, and it may take them some little time after birth to hypertrophy in response to the additional demands. It is thought that this would explain the finding of low urea clearances and isotonic, but not hypotonic urines.

(c) At birth certain parts of the human kidney are still undeveloped. According to Peter [1927] the glomeruli are all formed, but the nephrons attached to those near the capsule are still very primitive, and the loops of Henle very short. The most central nephrons are in a much more advanced stage of development, but even they have many primitive features. If the loops of Henle were functionally defective at birth, the kidney might be unable to reabsorb enough water to produce a hypertonic urine, and could then be described as being still in the stage of functional development characteristic of the fish and amphibia. Unfortunately any conclusions about function based upon measurements of the loops of Henle are complicated by the fact that even in the adult the loops are of very different lengths [Peter, 1909].

(3) Seeing that the infantile kidney is such a relatively ineffective organ, how comes it that it is able to do its job so satisfactorily?

There seem to be two answers to this question:

(a) Human milk is such a perfectly constituted food for the human infant, and the rate at which new body tissues are being laid down is so great, that for some time after birth the kidney has relatively little to do.

(b) The infantile kidney is only capable of maintaining a normally constituted serum when everything is going well. Ribadeau-Dumas, Segurier & Mignon [1937], for instance, showed that a minor change of diet may produce surprising changes in the blood urea, and in a small series of babies of 2-7 months, recently studied, the blood ureas were found to be higher in those which were being artificially fed, and therefore on a greater protein intake, than in those which were being breast fed. A study of infants' blood chemistry in diseases likely to throw even a mild strain upon their kidneys has revealed surprising departures from the normal. It is hoped to expand this aspect of the matter in a future publication.

(4) Have the present discoveries any therapeutic significance?

They certainly have, for:

(a) Since the clearances of inulin, urea, sodium and chloride have all been found to be very low in infancy but to increase with the minute volume, it is clearly important to keep the fluid intake at such a level that the output by the kidney is maintained. Oliguria in fact is synonymous with renal insufficiency.

(b) The results show how necessary it is to control the amount of protein in the diet, especially when the urine output is diminished during periods of pyrexia, or increased fluid loss by the gastro-intestinal tract.

(c) They emphasize particularly how carefully saline should be given to infants. Normal saline rightly enjoys a great reputation as a therapeutic agent, but its administration to adults requires care [Jones & Morgan, 1938], and, if the infantile kidney finds it difficult or impossible to excrete a hypertonic urine, then clearly normal saline ought to be placed on the list of dangerous drugs, only to be used after a careful consideration of dosage.

SUMMARY

An investigation of the kidney function of newborn infants has shown that:

1. Their minute volumes varied from 0.022 to 0.38 c.c. This corresponds very well with the adult range when both are expressed on a surface area basis.
2. Their urea clearances varied as a rule with the minute volume, but were always very low by adult standards whether compared on a basis of surface area, kidney weight, or body weight.
3. Their sodium and chloride clearances were very low by adult standards even when the plasma values were abnormally high.
4. Their potassium clearances were low although the serum potassiums tended to be high and were sometimes twice the normal adult level.
5. Their urines were always hypotonic and generally extremely so.

A consideration of 2, 3, 4 and 5 leads to the conclusion that the kidney of the very young infant is a relatively ineffective organ, the causes and significance of which have been discussed.

6. A supplementary investigation on three newborn infants with meningoceles has shown that their inulin clearances were of the order of 30 c.c./sq. m./min. at normal urine flows. These clearances varied with the minute volumes and were $2\frac{1}{2}$ -3 times the urea clearances. The ratio of the urea/inulin clearances did not alter consistently with the minute volume. Hence, it is concluded that in infancy the glomerular filtration

rate varies extensively with the minute volume but that the reabsorption of urea does not do so.

The authors are very grateful to Prof. L. G. Parsons for the facilities which he placed at their disposal; to Dr Frances Braid for allowing them to investigate her patients, and to the nursing staff of the Maternity Hospital, Loveday Street, Birmingham, for their help. They have also to thank Miss E. Tonks, M.Sc. and Miss E. Finch, M.Sc. for some valuable technical assistance. The expenses of the work were partially defrayed from a grant made by the Medical Research Council for research at the Children's Hospital, Birmingham. W. F. Y. is also indebted to the Council for a personal grant.

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BLOOD FLOW, ARTERIAL OXYGEN SATURATION, AND OXYGEN CONSUMPTION IN THE ISOLATED PERFUSED HINDLIMB OF THE DOG

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THE effective pressure head of oxygen between the blood and the muscle cells was held by Verzář [1912, 1916, 1920] to be one of the factors which determine the rate of metabolism of resting mammalian muscle. The essential evidence underlying this view was the finding that when the mean oxygen tension in the blood supplying the gastrocnemius muscle of the cat was diminished as a result of either spontaneous changes of blood flow or of experimentally produced changes in the oxygen content of arterial blood, then the oxygen consumption of the muscle, as measured by the product of the blood flow and the arterio-venous difference in oxygen content was also diminished.

The views expressed by Verzář have been supported and extended by the anatomical researches of Krogh [1919*a, b*, 1928], who regards the state of the capillary circulation as an essential factor regulating the oxygen supply to mammalian muscle. According to Krogh the number of open capillaries in resting muscle is insufficient to maintain a positive pressure of oxygen everywhere in the tissue, and under these conditions diffusion is the limiting factor regulating the oxygen supply. A diminution of the pressure head of oxygen between the capillaries and the muscle cells would then result in a diminished oxygen consumption and the experiments of Verzář have been interpreted by Krogh in this way.

Although no systematic investigations of the effects of changes of blood flow and of arterial oxygen saturation have followed the researches

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of Verzá, a number of investigators using similar methods have since made conflicting observations concerning the effects of changes of blood flow on the oxygen consumption of hindlimb preparations in the cat or dog. Nakamura [1921] reported that the oxygen consumption of the cat's hindlimb was unaffected by changes of blood flow "...except when the flow becomes very small". Freund & Janssen [1923] supported the findings of Nakamura and state that the critical value of blood flow below which the oxygen consumption is diminished is 0.05 c.c./g. muscle/min. In a few observations on the perfused hindlimb of the dog Rapport & Katz [1927] did not find a correlation between blood flow or arterial oxygen content and rate of oxygen consumption. On the other hand Rein & Schneider [1937], using the method of Kramer [1935] for the continuous registration of the oxygen saturation of the blood, found that the rate of oxygen consumption of the hindlimb of the dog was reduced during short periods in which the blood flow through the femoral artery was reduced by clamping. The decreased oxygen use during the period of clamping was, however, offset by an equal increased oxygen use after the original blood flow was restored. Cammer & Griffith [1939] have noted a general correspondence between the blood flow and the oxygen consumption of the hindlimb of the cat.

The relationship between the blood flow and the arterio-venous difference in oxygen content (abbr. AVO_2 -diff.) of mammalian muscle has recently been given a new significance, however, by the demonstration that a diminution of blood flow caused by either electrical or reflex stimulation of the vasoconstrictor nerves to the perfused hindlimb or gastrocnemius muscle of the dog may result in a decreased AVO_2 -diff., whereas a similar change of blood flow caused by the action of adrenaliné or by a reduction of arterial perfusion pressure results in an increased AVO_2 -diff. [Pappenheimer, 1941]. Whereas in the former case the oxygen consumption as calculated by conventional means is greatly reduced, in the latter case it may be relatively unaffected. Reasons have been given for supposing that the reduction in the product of blood flow and AVO_2 -diff., which occurs during vasoconstriction produced by nervous means, represents only an apparent change of oxygen consumption and that any real net change in oxygen use which may occur under such conditions may be attributed to other factors. It was mentioned in this connexion that even a change of blood flow caused by a sudden change of perfusion pressure may result in large, though transient, changes of "apparent" oxygen consumption—a phenomenon which will be described in more detail below.

It will be evident from the foregoing that the analysis of physical or chemical factors affecting the oxygen consumption of resting mammalian muscle from measurements of blood flow and AVO₂-diff. requires a careful control of variables of a physiological nature—in particular sympathetic activity and blood pressure.

It is the primary object of this paper to describe the effects on the rate of oxygen consumption of the isolated resting hindlimb of (a) changes of blood flow produced by changes of arterial perfusion pressure at constant arterial oxygen saturation, (b) changes of arterial oxygen saturation at constant blood flow.

It is a secondary object to give a more general picture than has been given previously of the metabolic and circulatory properties of the hindlimb under the conditions of our experiments.

METHODS

The pump-lung-hindlimb preparation was made as described previously [1941], with the exception that in later experiments negative pressure ventilation was used for respiring the lungs. The blood flow through the lungs is increased as a result of this procedure [Daly, 1928] and their efficiency in the oxygenation or de-oxygenation of the perfusing blood is also improved. The diffusion constant of oxygen for lungs perfused under these conditions is nevertheless only a small fraction of its value *in vivo*, and we have experienced difficulty in obtaining oxygen saturations of less than 25 % even when 95 % nitrogen (purified over hot freshly reduced copper) and 5 % carbon dioxide were used as the respiratory gas. At such low saturations the oxygen taken up by the blood at various open points in the perfusion circuit is apparently sufficient to offset the de-oxygenation in the lungs.

The oxygen saturations of arterial and venous bloods were measured and recorded continuously by the methods of Kramer & Winton [1939]. The earlier investigations for which this method has been used, and for which accurate quantitative measurements of the AVO₂-diff. were required; were made under conditions in which the AVO₂-diff. and the arterial saturation remained relatively constant [Kramer & Winton, 1939; Eggleton, Pappenheimer & Winton, 1940]. The extension of the method to meet conditions in which these variables are purposely varied over a wide range requires a more detailed analysis of the errors of the method than has been given previously. These errors, together with the precautions which have been adopted to minimize them, are described below.

(1) *Temperature.* Owing to the heat loss from the glass and rubber tubing of the perfusion system, and from the hindlimb itself, the temperature of the arterial blood supplying the photocell unit and the hindlimb and that of the venous blood supplying the photocell unit may vary by as much as 5°C. when large changes of blood flow occur as a result of changes in perfusion pressure. Errors of two types may arise from such variations:

(a) At constant oxygen saturation blood absorbs less light of all relevant wave-lengths (575–700 m μ .) at low than at high temperatures, and the slope of the calibration curve relating oxygen saturation to light transmission may be significantly altered by changes of temperature due to changes of blood flow [Eggleton *et al.* 1940]. Electrically heated silver tubes inserted at appropriate places in the perfusion system have been used to eliminate errors from this source [Pappenheimer, 1941].

(b) Although the temperature of arterial blood supplying the hindlimb is maintained constant (between 36.5 and 37.5°C. in these experiments) it is possible that the temperature in the tissues may be sufficiently altered by large changes of flow to affect the metabolism. A few estimates of the effects of temperature on the rate of oxygen consumption, under the conditions of these experiments, indicate a change of about 5% per 1°C. over the range 30–38°C. Assuming the mean temperature in the tissues to be half-way between that of arterial and venous bloods, then the error from this source may be as large as 15% at the lowest flows shown in Fig. 4.

(2) *Blood flow.* The absorption of light by flowing blood at constant temperature and saturation may vary with the rate of flow. The direction and magnitude of the variation is mainly determined by the properties of the tube used as absorption chamber. The chamber used in the flow experiments described in this paper was of flat fused glass 35 \times 10 mm. with a lumen 1 mm. thick, and the variation in the transmittance of light with a change of flow 10–200 c.c./min. was ordinarily equivalent to a change of about 1% oxygen saturation. The error introduced into the determination of the AVO_2 -diff. is, however, less than this, for both arterial and venous bloods are measured in the same absorption chamber at the same flow. Below 10 c.c./min. the rate of settling of the red blood corpuscles may become a significant factor, tending to increase the transmittance of light. In one experiment, in which the red cells settled with exceptional rapidity, this error became appreciable at a flow of 30 c.c./min.

(3) *Departure of the calibration curve from linearity.* Over the range of oxygen saturations 60–100%, the oxygen combined with haemoglobin is a linear function of the logarithm of the galvanometer deflexion within the standard error of the calibration methods [Pappenheimer, 1941]. At oxygen saturations below 60%, however, the calibration curve may depart from linearity, under the conditions of these experiments, as shown in Fig. 1. There are several possible causes for this departure, which are at present under investigation. It is our impression that the departure may be reduced by partly eliminating from the light source wave-lengths greater than 700 m μ . (Corning 430 glass filter, $\frac{1}{4}$ standard.)

(4) *Dissolved oxygen.* At high values of blood flow and arterial oxygen saturation, a considerable fraction of the AVO_2 -diff. of the hindlimb may be in the form of dissolved oxygen. Since the transmission of light depends upon the combined oxygen, a serious error in the measurement of the true AVO_2 -diff. may arise. Corrections for this error may be applied if the oxygen dissociation curve of the blood is known. We have applied such corrections in all cases where they appeared significant, using the dissociation curve of dog's blood at 40 mm. CO_2 pressure obtained by Bohr [1909]. Examples of the magnitude of such corrections for various blood flows and oxygen saturations are seen in Table I. Although the gas mixtures with which the lungs were respired always contained 5% carbon dioxide, there is little assurance that the pH of the blood remained constant and equivalent to that of normal dog's blood at 40 mm. CO_2 pressure. A second-order error in the calculation of the dissolved oxygen may therefore be present and may be responsible for the greatest error in the measurement of AVO_2 -diff. at high blood flows and saturations.

(5) *Errors of timing.* Owing to the dead space in the glass and rubber tubing and in the large veins a certain time elapses before venous blood from the tissues reaches the photocell unit and before arterial blood passing the photocell reaches the tissues. In analysing the records a correction is made whose value is determined by the dead space

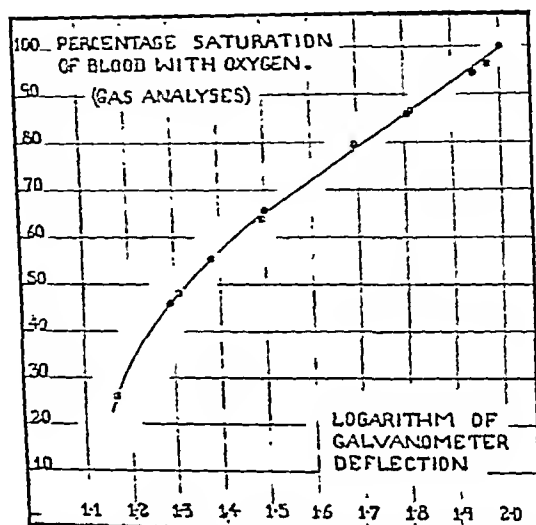


Fig. 1. A calibration of the oxygen recording unit. Experiments 12A and 16A. Temperature 37° C. Blood flow 100 c.c./min. Corning 430 glass filter, $\frac{1}{4}$ standard. Gas analyses by manometric method of Van Slyke & Neill [1924].

TABLE I. Errors in measurement of AVO_2 -diff. due to dissolved oxygen

Blood flow c.c./min.	Arterial oxygen content			Venous oxygen content			AVO_2 -diff. vols. %	Percentage of AVO_2 -diff. due to dissolved oxygen
	Com- bined vols. %	Per- centage satura- tion	Dis- solved vols. %	Com- bined vols. %	Per- centage satura- tion	Dis- solved vols. %		
179	20.45	99.4	0.45	19.72	95.7	0.29	0.89	17
70	20.48	99.5	0.45	18.82	91.3	0.23	1.83	12
12	20.45	99.4	0.45	14.38	89.7	0.14	6.38	5
105	11.80	57.3	0.12	11.02	53.6	0.17	0.79	1

and the blood flow. Thus in the experiment shown in Fig. 3 the dead space was about 10 c.c., so that at a blood flow of 100 c.c./min. the oxygen saturation of the venous blood at any point in the record was considered to represent the saturation of the blood in vein 6 sec. previously. Under rapidly changing conditions of blood flow and oxygen saturation this correction becomes increasingly important in the calculation of the product of the blood flow and the AVO_2 -diff. at any moment. Corrections of a similar nature are made for the curvatures of the recording levers.

VARIATIONS IN OXYGEN CONSUMPTION AT CONSTANT
BLOOD FLOW AND ARTERIAL OXYGEN SATURATION

In order to assess the significance of changes of oxygen consumption which may follow alterations of blood flow or of arterial oxygen saturation, a knowledge of the variations encountered when these variables are held constant is necessary.

The blood flow and the arterial oxygen saturation may ordinarily be maintained constant within 1% for periods of several minutes, and under these conditions there may be no detectable change in AVO_2 -diff. Over periods of several hours, however, such constancy is difficult to obtain, and in order to estimate the variations of oxygen consumption which may occur during 4 or 5 hr. of perfusion the following somewhat arbitrary procedure has been employed. The rate of oxygen consumption is measured at intervals under conditions in which percentage saturation of arterial blood does not differ by more than 3 from a given value and the blood flow is greater than 60 c.c./min. and within 20% of any given value. The standard deviation from the mean of all the values obtained is then considered as the variation to be expected under the arbitrarily selected conditions. Data from a typical experiment are shown in Table II. In fourteen such experiments the standard deviation of the rate of oxygen consumption from the mean value was 7.5% and the maximum variation was 25.6%. In some experiments curarine was added so that muscular activity due to irritation of the cut nerve could not contribute to the variations of oxygen consumption. No significant difference between the curarized and the non-curarized preparation was observed, however.

TABLE II. Variations of oxygen consumption under constant conditions

Exp. no.	Time perfused hr. and min.	O ₂ - capacity vols. %	Arterial saturation %	Blood flow c.c./min.	O ₂ -con- sumption c.c./min.	Mean	Deviation %
VI	0.10	—	98	60	1.96	—	10.3
9. xii. 38	1.00	—	96	70	1.70	—	4.6
	2.00	23.44	96	87	1.75	• 1.78	1.7
	3.30	—	99	83	1.74	—	2.3
	4.30	23.35	98	75	1.73	—	2.8

It is worth noting that the average AVO_2 -diff. in these observations was 1.9 ± 0.6 vols. %, so that a change of oxygen consumption of 7.5% corresponds with a change of 0.14 ± 0.04 vols. % in the AVO_2 -diff. Although a change of this magnitude is too small to be detected with confidence by gas analysis, it may well be a significant one, for it

corresponds with a change of approximately 5 mm. in the galvanometer deflexion, which is ordinarily constant within 2 mm. There is some evidence that the variations are not random, but are due to a slow progressive drift in the oxygen consumption during the course of the perfusion. Thus the mean oxygen consumption during the 3rd and 4th hr. of perfusion under the conditions specified above was $7.6 \pm 5.5\%$ lower than during the first 2 hr. of perfusion.

THE EFFECTS OF CHANGES OF BLOOD PRESSURE ON THE BLOOD FLOW AND THE OXYGEN CONSUMPTION AT CONSTANT ARTERIAL OXYGEN SATURATION

A. *Transient changes.* The immediate effects of a sudden rise of arterial pressure on the blood flow and AVO_2 -diff. of the isolated hindlimb may vary considerably both from one preparation to another and in the same preparation during the course of an experiment. The types of responses which have been observed are illustrated in Fig. 2*a* and *b* and in Fig. 3. They appear to depend upon what may be called the "vascular reactivity" of the limb. In those preparations in which the reactivity is great a rise of pressure is associated with a vasodilatation lasting some 30–60 sec. (Fig. 2*a*). This may be associated with an increased AVO_2 -diff., as indicated by the decrease in the oxygen content of venous blood (Fig. 2*b*). The phenomenon may be simulated by the intra-arterial injection of small doses of histamine, as shown in Fig. 2*c*, or by stimulation of the vasodilator nerves [Pappenheimer, 1941]. Rein & Schneider [1937] have noted similar changes in the blood flow and AVO_2 -diff. of the intact limb following the release of a clamp on the femoral artery. In the isolated hindlimb or gastrocnemius preparation the phenomenon has only been observed early in experiments in which the transfer from the natural to the artificial circulation has been particularly swift. One forms the impression, therefore, that "vascular reactivity" is a property of the normal limb which is lost as perfusion proceeds.

The effects of changes of blood pressure in the non-reactive limb are illustrated in Fig. 3. It will be noted that even in this case the blood flow and the AVO_2 -diff. do not reach their equilibrium values at the same rate and small changes of apparent oxygen consumption may occur.

B. *Equilibrium values.* The equilibrium values of rates of oxygen consumption over the range of blood flows 8–190 c.c./min. caused by changes of perfusion pressure are shown in Fig. 4. Although the effects of changes of perfusion pressure have been measured in some forty

hindlimb or muscle preparations, the data depicted in Fig. 4 are from four of the later experiments which have had the benefit of all the

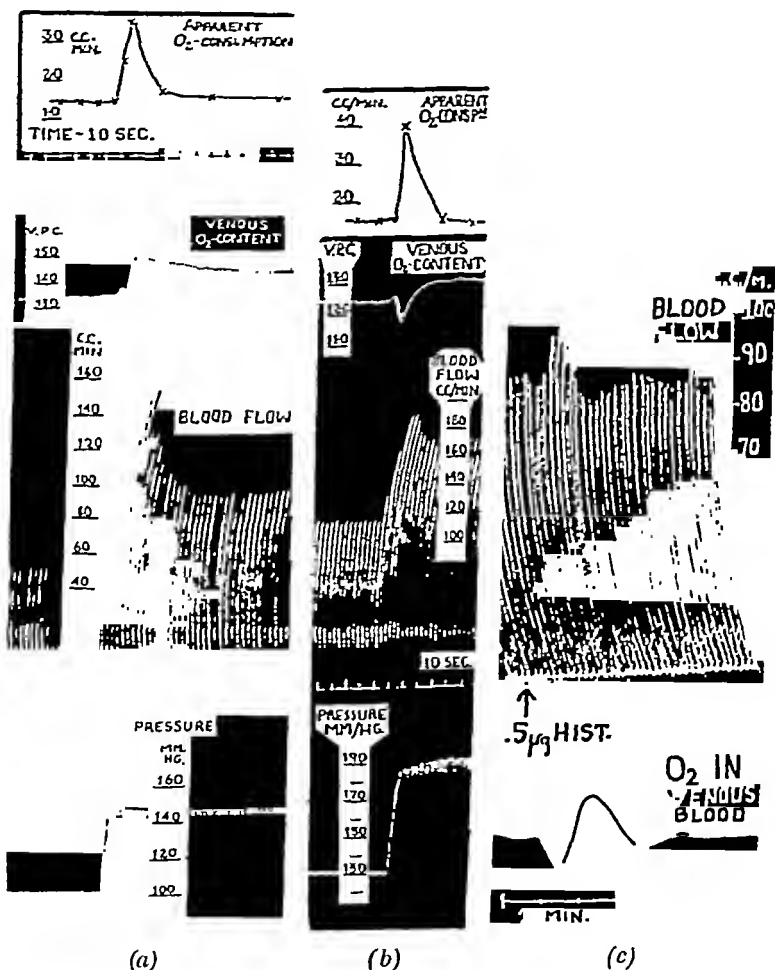


Fig. 2. The effect of a sudden rise of perfusion pressure on the blood flow and on the venous oxygen content in the "reactive" hindlimb. (a) Transient vasodilatation and increase of apparent oxygen consumption. (b) Transient vasodilatation accompanied by a fall in venous oxygen content. (c) Changes of blood flow and of venous oxygen content similar to those shown in (a) and (b) but elicited by the injection of 0.5 μ g. histamine phosphate into arterial cannula.

precautions mentioned above under Methods. The oxygen calibration curve of one of these experiments is shown in Fig. 1. The general effect of increasing technical experience has been to shift the oxygen consumption

blood flow curve slightly to the left; if all the experiments were considered, the mean value of blood flow at which the oxygen consumption begins to decline is nearer to 60 than to 40 c.c./min.

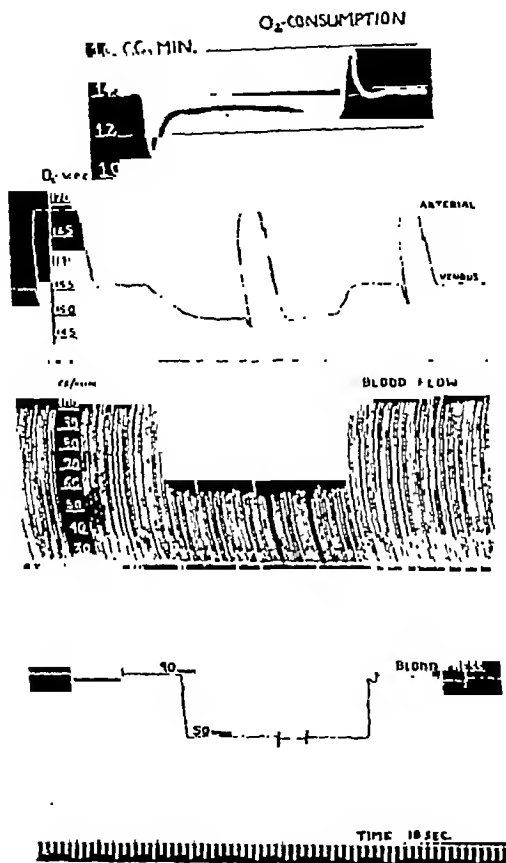


Fig. 3. The effects of changes of perfusion pressure on the blood flow and on the venous oxygen content in the "non-reactive" hindlimb. Although vasodilatation does not occur when the pressure is raised suddenly as in the reactive limb (Fig. 2) the blood flow and the venous oxygen content nevertheless reach their equilibrium values at different rates resulting in changes of apparent oxygen consumption.

The maximum variations in the calculated rates of oxygen consumption at any given blood flow are enclosed within the area which has been "cross-hatched", and the mean rate of oxygen consumption is given by the centre curve.

In order that an approximate idea of the blood pressure corresponding to each of the blood flows shown may be obtained, a typical pressure-flow curve taken from one of the experiments is shown in the inset (Fig. 4)

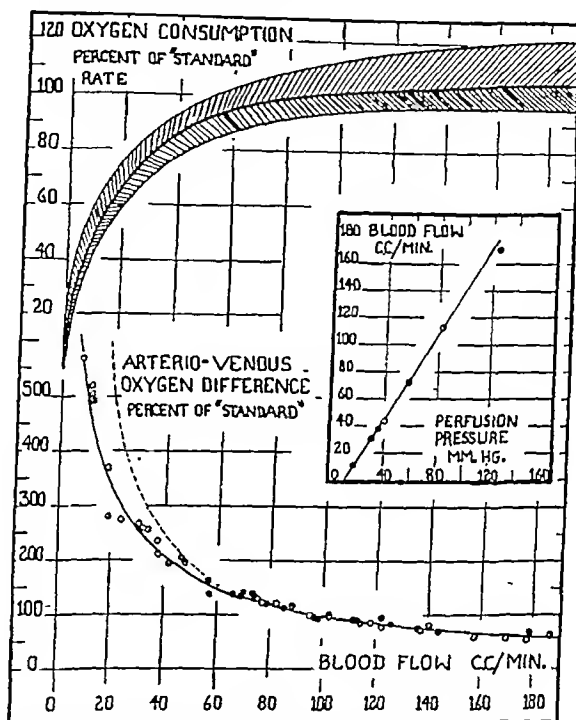


Fig. 4. The effects of changes of blood flow caused by changes of perfusion pressure on the arterio-venous oxygen difference and on the rate of oxygen consumption. The cross-hatched area encloses the maximum variations in the rate of oxygen consumption at any flow as calculated from the observed AVO_2 -diff., and the centre curve represents the mean values. The broken line indicates the AVO_2 -diff. which would have obtained had the oxygen consumption remained at its "standard" (100%) value. The inset shows a typical pressure-flow curve. For reasons which have been given in the text, it is only an approximate guide to the pressures corresponding to the blood flows. The absolute values of AVO_2 -diff., venous oxygen saturations, and rates of oxygen consumption may be calculated roughly from data given in the text. Temperature 36.5-37.5°C. Arterial saturation greater than 90% and constant to $\pm 2\%$. "Standard" rate of oxygen consumption measured at a blood flow of 100 c.c./min.

Owing to changes of vascular tone during the course of an experiment, however, considerable variation from any one pressure-flow curve may occur as described below.

The average value of the absolute rates of oxygen consumption of the four hindlimbs at a blood flow of 100 c.c./min. (arterial saturation

greater than 90% and constant to $\pm 2\%$) was 1.7 ± 0.4 c.c./min. and the average oxygen capacity of the bloods used for perfusion was 17.6 ± 1.5 vols. %.¹ From these values the absolute values of rates of oxygen consumption, AVO₂-diff. and venous saturation corresponding to any given blood flow may be roughly calculated.

For reasons stated above under Methods, reliable measurements of the AVO₂-diff. at blood flows of less than 8 c.c./min. were not obtained. Evidence exists, however, which suggests that even if the blood flow were allowed to approach zero, the venous blood would still retain some of its oxygen. Such evidence has come from experiments in which the circulation was clamped off entirely for varying periods and the minimum venous oxygen saturation recorded when the circulation was restored. Data from a typical experiment are shown in Table III. It is seen that

TABLE III. Minimum venous oxygen saturations

Period of clamping min.	Minimum venous O ₂ saturation during recovery %	Calculated tension in venous blood mm. Hg
2	37	23
5	16	14
10	2*	3

* This value was estimated from the oxygen calibration curve (similar to that shown in Fig. 1) by extrapolation. Had the saturation been greater than 2% then the calibration curve must have had a point of inflexion between 0-26% saturation.

the venous blood was still 16% saturated even after a 5 min. period of clamping, but that almost complete utilization of oxygen from the blood did occur after 10 min. This observation is of interest, for it shows that the intracellular oxidative processes in resting mammalian muscle can continue to operate at extremely low tensions although the rate of oxygen consumption may be diminished.

The critical value of blood flow at which the rate of oxygen consumption started to decline in the experiments shown in Fig. 4 was about 40 c.c./min., and at this flow the AVO₂-diff. averaged 3.7 ± 0.7 vols. % and the oxygen saturation and the calculated oxygen tension in the venous blood averaged $79 \pm 7\%$ and 52 ± 7 mm. Hg respectively. The significance of these values will be considered in the discussion.

¹ This value is lower than usual. The average oxygen capacity of blood from 103 English dogs used in these and other perfusion experiments has been 21.6 ± 2.7 vols. % and of blood from twelve Pennsylvania dogs has been 20.6 ± 2.6 vols. %.

THE EFFECTS OF NON-NERVOUS CHANGES OF VASCULAR TONE ON THE
BLOOD FLOW AND OXYGEN CONSUMPTION AT CONSTANT
ARTERIAL PRESSURE AND OXYGEN SATURATION

The absolute value of the blood flow through the isolated hindlimb at any given perfusion pressure may be considerably greater than that in the intact limb at the same pressure, provided precautions have been taken to free the blood from the vasoconstrictor substances present in the shed blood (i.e. "detoxication" in the lungs). Thus in our series of experiments on the hindlimbs of dogs weighing 9-16 kg., the blood flow at 100 mm. perfusion pressure during the first 2 hr. of perfusion has varied from 60 to 200 c.c./min./10 kg. dog, with a mean value of 110 c.c./min. Data from sixteen of these experiments, in which figures for the absolute value of oxygen consumption under specified conditions were also obtained, are shown in Table IV. Comparable figures for the

TABLE IV. Absolute values of blood flow and oxygen consumption—hindlimbs*

Exp. no.	Weight of dog kg.	O ₂ -consumption† of limb c.c./min.	O ₂ -consumption per 10 kg. dog c.c./min.	Blood flow‡ per 10 kg. dog c.c./min.
7	12	1.8	1.5	115
10	16	2.2	1.4	60
13	10.5	1.4	1.3	100
15	15	1.6	1.1	70
16	14.5	2.6	1.8	90
17	10	1.4	1.4	110
20	12	2.7	2.2	115
22	9	1.6	1.8	—
2A	13	1.9	1.5	95
3A	12.5	1.9	1.5	130
4A	10	2.3	2.3	150
15A	10.5	1.2	1.1	110
16A	10	1.2	1.2	80
17A	14	1.7	1.2	95
18A	9.5	1.6	1.7	140
19A	9	1.3	1.4	140

Mean 1.5 ± 0.4 Mean 107 ± 25

* In other experiments on the pump-lung-gastrocnemius preparation the oxygen consumption and blood flow measured under similar conditions averaged 0.009 c.c./min./g. muscle and 0.75 c.c./min./g. muscle respectively. Both these values suggest that the weight of muscle perfused in the "hindlimb" preparation is about 150 g./10 kg. dog. The measured weight of the preparation including bone but not the skin has been found to be about 250 g./10 kg. dog.

† Oxygen consumption measured at blood flow of 100 c.c./min. and arterial oxygen saturation greater than 90% and constant.

‡ Blood flow measured at pressure of 100 mm. between 1st and 2nd hr. of perfusion.

blood flow through the femoral artery and vein in the intact hindlimb at 100 mm. pressure have been given by Keller, Loeser & Rein [1930], who report a variation of 14-45 c.c./min. In no case have they reported a flow greater than 60 c.c./min. even following denervation.

The blood vessels of the isolated preparation nevertheless possess a considerable tone of their own which is capable of being released by the mechanisms usually described for the intact limb, and the blood flow may vary considerably during the course of an experiment even though the blood pressure is maintained constant. Some of the factors influencing these variations have been discussed by Whittaker & Winton [1933]. It is of importance to determine the effect on the oxygen consumption of such variations in blood flow due to changes of vascular tone of non-nervous origin, for there has been no clear distinction in the literature

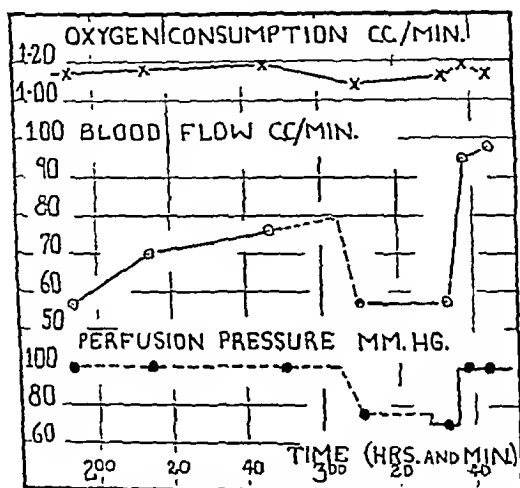


Fig. 5. The effects of a "spontaneous" decrease in non-nervous vascular tone on the blood flow and on the rate of oxygen consumption. Semi-diagrammatic in that between each observation shown in graph several measurements of blood flow and oxygen consumption were made at different pressures. Temperature 36.5° C. Arterial oxygen saturation 93.5 ± 1% throughout.

between non-nervous vascular tone and tone of sympathetic origin. The characteristic effects on the apparent oxygen consumption and on the arterio-venous difference of temperature afford new criteria for making such a distinction, and it has already been shown that equal changes of blood flow at constant pressure caused by adrenaline on the one hand, and by the vasoconstrictor nerves on the other, have opposite effects on some of the properties of venous blood [Pappenheimer, 1941].

Fig. 5 illustrates an experiment in which a "spontaneous" decrease in vascular tone occurred, as indicated by the increasing blood flow at constant pressure. In spite of the increase in flow from 57 to 98 c.c./min. at a pressure of 100 mm. Hg the rate of oxygen consumption was not

significantly changed. A substantial *increase* of vascular tone has only been observed in one experiment, data from which are shown in Table V.

TABLE V. The effects of a spontaneous increase of vascular tone

Time perfused hr. and min.	Perfusion pressure mm./Hg	Blood flow c.c./min.	Arterial saturation %	O ₂ -consumption c.c./min.
0.45	50	105	98	2.29
3.38	90	93	95	2.33
1.15	70	134	96	2.55
1.30	44	53	98	2.00
3.01	70	58	96	2.16

It would therefore appear that changes of blood flow due to spontaneous changes of vascular tone, unlike nervous changes, affect the oxygen consumption in a way indistinguishable from similar changes of blood flow brought about by changes of perfusion pressure.

OXYGEN SATURATION AND OXYGEN CONSUMPTION AT CONSTANT BLOOD FLOW

Fig. 6 illustrates a typical experiment in which the oxygen saturation of the blood was altered by respiring the lungs in the perfusion circuit with gas mixtures containing 95% N₂+5% CO₂, air+5% CO₂, or O₂+5% CO₂. It is seen that there is a close qualitative relation between the rate of oxygen consumption and the arterial saturation. Quantitatively, however, the relation is not so clear. It will be noticed that at any given arterial saturation the oxygen consumption tends to be lower when the arterial saturation is diminishing than when it is increasing. For example, at 6.08 the oxygen consumption was 1.20 c.c./min., whereas at 6.28 it was 1.79 c.c./min. although the arterial saturation was 87% in both cases. Although the errors in the measurement of AVO₂-diff. under such rapidly changing conditions are increased and are difficult to estimate, as discussed above under Methods, the differences have been sufficiently consistent to suggest that the increased oxygen consumption at any given saturation when the saturation is increasing may be due to the paying off of an oxygen debt incurred at the low saturations. That resting muscle, under the conditions of these experiments, may pay off at least a part of the oxygen debt incurred during a complete arrest of the blood flow has been shown in a previous communication [Pappenheimer, 1941].

In analysing further the effects of changing the arterial oxygen saturation, we have attempted to obtain "equilibrium" values of oxygen consumption at various saturations. In such experiments the lungs were

respired with suitable gas mixtures until the required saturation of the blood was obtained. Ventilation of the lungs was then stopped; owing to the large volume of blood, now partially de-oxygenated, in the system, the saturation of both arterial and venous blood then ceased to change appreciably. This procedure has the disadvantage that carbon dioxide accumulates in the blood during the period in which respiration is

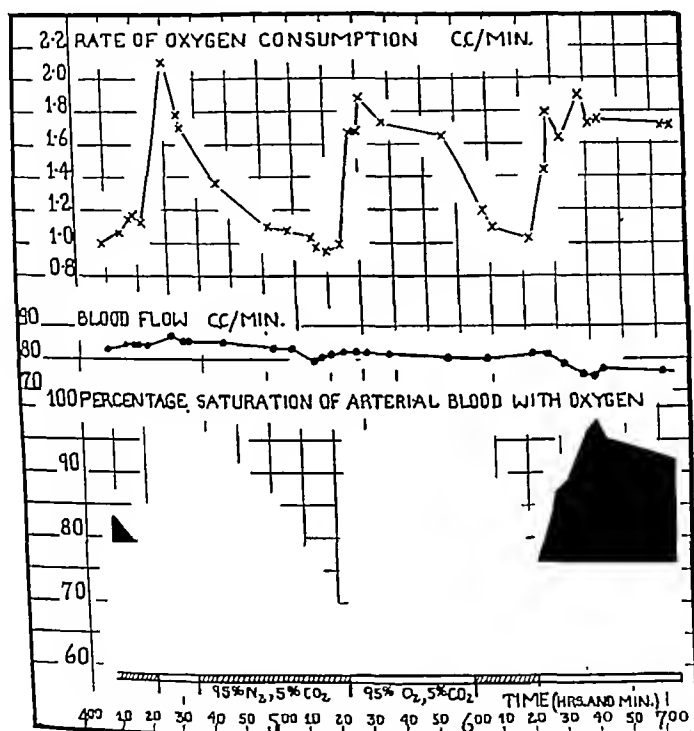


Fig. 6. Arterial oxygen saturation and oxygen consumption at constant blood flow. Compare with equilibrium values given in Table VI.

stopped, thus introducing a new variable which may affect the oxygen dissociation curve. The maximum change of CO₂ content which has been observed to result from this procedure was 13 vols. %, a change corresponding to about 25 mm. in the CO₂ tension. An increase of CO₂ tension from 40 to 65 mm. Hg would have the effect of shifting the oxygen dissociation curve 5-7 mm. to the right over the range 20-60% saturation [Bohr, 1909]. In calculating the oxygen tensions corresponding to the observed saturations, therefore, small corrections have been made for

changes in CO_2 content as determined by gas analysis. Such corrections have only appeared significant in the two lowest tension groups shown in Table VI (40–20 mm. Hg).

Table VI summarizes the data obtained in nine experiments in which either equilibrium values were obtained or in which the oxygen consumption was measured during a slow decline in arterial oxygen saturation.

TABLE VI. Oxygen tension and oxygen consumption

Oxygen tension* in venous blood mm. Hg	No. of experiments	No. of observations	O_2 -consumption† mean, standard deviation and standard error of mean
80–70	4	4	101 ± 2 (± 1.0)
70–60	6	17	97 ± 6 (± 1.4)
60–55	6	8	81 ± 16 (± 5.6)
55–50	5	7	71 ± 16 (± 6.0)
50–40	8	30	64 ± 9 (± 1.6)
40–30	7	13	57 ± 9 (± 2.5)
30–20	2	7	66 ± 7 (± 2.7)

* Calculated from the observed venous oxygen saturations and the oxygen dissociation curves of dog's blood obtained by Bohr [1909].

† In percentage of that at an arterial saturation of 95% and a blood flow greater than 70 c.c./min.

In analysing the results we have chosen to consider the tension of oxygen in the venous blood as the variable rather than the arterial tension or saturation or the averages of the arterial and venous values. This choice was made mainly because we wished to obtain a minimum figure for the oxygen tension at which the "standard" rate of oxygen consumption could be maintained. It will be evident that the results would differ quantitatively but not qualitatively if one of the alternative methods of presentation had been chosen.

It is seen that when the rate of oxygen consumption is reduced significantly (mean reduction greater than twice the standard error of the mean) the tension of oxygen in the venous blood lies between 60 and 55 mm. Hg (85–81% saturated).

DISCUSSION

In considering possible reasons for the diminished rate of oxygen consumption of the resting perfused hindlimb when the blood flow or the oxygen saturation of the blood are reduced below certain critical values, two possible mechanisms or their combination come to mind.

(1) That the state of the capillary circulation and the value of the diffusion coefficient of oxygen through the tissues are such that all the tissues cannot be supplied with oxygen when the tension of oxygen in

the capillary blood falls below a certain value. This is the hypothesis which has been advanced by Krogh [1928].

(2) That diffusion is not, or is only in part, a limiting factor. This would imply that there exists a finite pressure of oxygen everywhere in the tissue and that the intracellular oxidative processes are directly inhibited by the fall of oxygen tension.

(1) The critical value of capillary oxygen tension below which all of the muscle cannot be supplied with oxygen was estimated by Krogh to be about 50 mm. Hg in resting muscle in which the open capillaries are relatively few in number (31 per mm.²). This value agrees well with the minimum (i.e. venous) critical tensions found in our experiments, which have been 52 ± 7 mm. Hg in the case of the blood flow experiments and 60–55 mm. Hg in the case of the arterial saturation experiments. It would be expected on Krogh's hypothesis, that if more capillaries were caused to open up, then the pressure head of oxygen required to supply all of the tissues would be diminished. In active muscle containing large numbers of open capillaries (2500 per mm.²), Krogh has estimated the critical pressure head to be less than 5 mm. Hg instead of 50 mm. Hg even though the rate of oxygen consumption was assumed to have been increased twenty-fold as a result of activity. If a similar number of open capillaries was present during rest, the calculated capillary oxygen tension necessary to supply the whole tissue would fall to 0.2 mm. Hg, and in this case the oxygen supply to the tissues could reasonably be expected to be independent of the rate of diffusion of oxygen.

We have tried experiments designed to test this expectation in three preparations to which histamine was added during changes of oxygen consumption caused by low blood flows or low arterial saturations. In none of the experiments did the increased blood flow which followed the infusion of histamine at constant perfusion pressure result in a change of oxygen consumption which was significantly different from a similar change of blood flow caused by a change of perfusion pressure. The results of one of these experiments are shown in Table VII. It is seen that the rate of oxygen consumption was, if anything, diminished rather than increased, and that the vasodilatation—and presumably it was a capillary dilatation—did not prevent the decreased rate of oxygen consumption which occurred when the oxygen saturation of the arterial blood was reduced.

It would, therefore, seem that an explanation of the diminished rate of oxygen consumption, in which diffusion alone is considered as the limiting factor, is not without objection.

TABLE VII. Oxygen consumption during histamine dilatation

Time perfused hr. and min.	Perfusion pressure mm. Hg	Arterial saturation %	Blood flow c.c./min.	O ₂ -consumption percentage of standard
0.35	62	92	76	96
2.02	80	88.5	96	98
2.10	0.5 mg. histamine added to perfusion reservoir*			
2.18	80	88	132	86
2.36	80	95	130	90
2.39	80	96	130	93
2.51	80	86	128	79
2.56	80	76	126	67
3.26	70	95	101	102

* Histamine phosphate. Volume of perfusion blood ca. 1 l.

(2) It was an assumption implicit in the calculations of Krogh and of Verzár that the intracellular oxidative processes were themselves unaffected by changes of oxygen tension, and that so long as a finite pressure existed within the muscle cells the rate of oxygen consumption would remain unchanged. A similar assumption has been made by Meyerhof [1930] and others in considering the factors affecting the rate of diffusion of oxygen into isolated tissues.

There is little decisive evidence, of which we are aware, to justify such an assumption in resting mammalian muscle. Even in the case of isolated cells the subject would appear to be a controversial one, for it has been found by Kempner [1937] that the respiration of various cells may be greatly affected by the oxygen tension of the environment, and that the relation between oxygen tension and oxygen consumption may be influenced by the composition of the fluid in which the cells are suspended. A review of the literature appertaining to isolated cells may be found in Kempner's [1937] paper.

In the case of active mammalian muscle, it has been shown by Hilton & Eichholz [1924] that the oxygen consumption of the working heart may remain unchanged when the oxygen saturation of the coronary blood is only 12%—a saturation corresponding to a tension of 12 mm. Hg. In the case of resting mammalian muscle, however, the evidence is less conclusive. Millikan [1937] has estimated the oxygen tension within the muscle cells of the soleus muscle of the cat from measurements of the oxygen saturation of the muscle haemoglobin. Under resting conditions the muscle haemoglobin was found to be fully saturated, so that the intracellular oxygen tension was presumably greater than 25 mm. Hg. When the blood supply was clamped off the oxy-muscle-haemoglobin was found to undergo reduction at a measurable rate until it reached a saturation of about 50%. This value would correspond to an intracellular

oxygen tension of about 2-3 mm. Hg. It might be inferred from the initial "constant" rate of reduction that the resting oxygen consumption was unchanged even at tensions below 10 mm. Hg. There are, however, several objections to such an inference:

(a) The true resting rate of oxygen consumption was unknown. It may be that when the intracellular oxygen tension is lowered sufficiently to cause a reduction in the oxygen saturation of the muscle haemoglobin, the rate of oxygen consumption has already been diminished.

(b) As has been pointed out to me by Dr Millikan, the assumption that the relation between the galvanometer deflexion and the oxygen saturation is a linear one is as yet without experimental verification under the conditions of his experiments.

(c) Even supposing the slope of the record to be a linear measure of the rate of oxygen consumption it would be difficult to decide within an accuracy of 20% the value of the slope at any moment. The total decrease in the rate of oxygen consumption which we have observed to occur when the tension of oxygen in the venous blood is reduced to 20-30 mm. Hg is about 40%.

(d) The oxygen dissociation curve of muscle haemoglobin within the cell (from which the tensions might be calculated) is unknown, although reasons have been given [Millikan, 1939] for supposing that the oxygen dissociation curve of muscle haemoglobin, unlike that of blood haemoglobin, may not be greatly different within the cell from without.

Until further evidence becomes available, therefore, both the rate of diffusion of oxygen and a direct effect on the intracellular oxidative processes may be regarded as possible factors responsible for the decreased rate of oxygen consumption of the isolated hindlimb at low blood flows and oxygen saturations.

SUMMARY

1. At constant blood flow and arterial oxygen saturation the oxygen consumption of the isolated perfused hindlimb of the dog may vary less than 10% over periods of 4 hr. During shorter periods the variation may be less than 3% (Table II).

2. The immediate effect of a sudden rise of perfusion pressure is to cause an increase in the product of blood flow and arterio-venous difference (apparent oxygen consumption). The pressure rise may result in a vasodilatation and an *increased* arterio-venous oxygen difference lasting some 20-60 sec. The phenomenon may be simulated by the intra-arterial injection of histamine (Figs. 2, 3).

3. The equilibrium effects of changes of blood pressure at constant arterial oxygen saturation depend upon the absolute value of the blood flow. Above 40 c.c./min. the rate of oxygen consumption is relatively independent of the blood flow. Below about 40 c.c./min. the rate of oxygen consumption is decreased—reaching 50% of its normal value when the blood flow is reduced to about 10 c.c./min.

4. Almost complete utilization of the oxygen in the blood may occur after the circulation has been arrested for 5–10 min. (Table III).

5. The effects of changes of blood flow due to non-nervous changes of vascular tone at constant pressure and arterial oxygen saturation are shown to be indistinguishable from similar changes of blood flow caused by changes of perfusion pressure. The distinction, in this respect, between non-nervous and nervous changes of vascular tone is discussed.

6. The rate of oxygen consumption may be reduced when the arterial oxygen saturation is reduced at constant blood flow. The oxygen tension in the venous blood when the rate of oxygen consumption is first significantly reduced is 60–55 mm. Hg.

7. Possible causes of the diminished rate of oxygen consumption when the blood flow or oxygen saturation are reduced below certain critical values are discussed. It is concluded that until further evidence becomes available, both the rate of diffusion of oxygen and a direct effect on the intracellular oxidative processes may be regarded as possible factors involved.

I wish to thank Prof. F. R. Winton for his constant help. It is a pleasure also to thank Prof. H. C. Bazett for his hospitality in enabling the work to be completed in his laboratory and to thank Dr G. A. Millikan for his interest and criticism.

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CHARACTERISTICS OF THE CIRCULATION OF HYPERTENSIVE RABBITS

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LITTLE work has been done to determine the characteristics of the peripheral circulation in the hypertension which, as Goldblatt, Lynch, Hanzal & Summerville [1934] found, follows partial constriction of the renal artery. Pickering & Prinzmetal [1938] showed that in rabbits the rise of blood pressure which follows a needle prick is no greater in hypertensive animals. Verney & Vogt [1938] found that in dogs with hypertension of renal origin the pressor response to intravenous injections of tyramine was increased, and that the response to adrenaline was sometimes increased. Our own preliminary experiments [Brown, McLean & Maegraith, 1939] revealed a heightened pressor response to tyramine and posterior pituitary extract in hypertensive rabbits.

The present study was a more thorough attempt to find whether the circulatory system responds abnormally to stimuli of several types after the production of hypertension. Also, the opportunity was taken to compare the responses of animals hypertensive as a result of partial constriction of the renal artery with those of animals hypertensive following the induction of glomerulonephritis.

METHODS

Production of hypertension. The method of constricting the renal arteries was that of McLean & Maegraith [1939]. A modification of the method of Masugi [1934] was used to induce glomerulonephritis. Nephrotoxic serum was prepared by injecting guinea-pigs intraperitoneally with twenty-five 5 c.c. doses of a 20% suspension of rabbit renal cortical tissue at 4-day intervals. The serum was absorbed with a suspension of washed rabbit blood cells, and complement destroyed by heating at 56° C. for 30 min. The rabbits received one or two 2.5 c.c. doses of this serum

intravenously, and this resulted after 1-4 days in increases in blood pressure which were maintained, in some cases irregularly, for periods varying from 5 weeks to at least 2 months.

Blood pressure. The blood pressure was measured by the carotid loop method [van Leersum, 1911]. Only animals with very soft pliable loops were chosen in order that the blood pressure could be determined quickly and accurately. It was found possible to get dependable readings every 5 sec.

Measurement of pressor responses. To measure the response to a given drug, the animal was anaesthetized with nembutal, except in the case of rabbit 3074. The injection was made into the marginal vein of the ear, and the experiment repeated at least once. Only one drug was used during the one period of anaesthesia. The increase in blood pressure was found by subtracting the average pressure for the 2 min. before from the average of the three highest readings after the injection. A decrease was determined in a comparable manner. The differences between the measured responses to the same dose of a drug averaged less than 2 mm. Hg, except in the case of posterior pituitary extract when the difference was 3.2 mm. Hg. The following drugs were used: adrenaline (liq. adren. hydrochlor., B.P., British Drug Houses, Ltd.), 0.6-2.5 $\mu\text{g./kg.}$; acetylcholine (acetylcholine hydrobromide, British Drug Houses, Ltd.), 0.25-0.5 $\mu\text{g./kg.}$; tyramine (tyramine-HCl, Brady and Martin, Ltd.), 0.5 mg./kg.; and posterior pituitary extract ("Infundin", Burroughs Wellcome and Co.), 0.03-0.12 unit/kg.

RESULTS

Multiple experiments were done on twelve rabbits before and after constriction of the renal artery, and on four rabbits given serum. The response to the pressor drugs was increased during the period of hypertension in all cases but two (Tables I-IV); one exception was apparent, the other real. The increased response was observed as soon as 10 hr. after operation. There was no strict relation between the ratios (normal response)/(hypertensive response) and (normal B.P.)/(hypertensive B.P.), but once the hypertension was established, the response varied roughly as the blood pressure. In a given animal the degree of increase was not the same for all three drugs. When the blood pressure had returned to normal, the pressor responses returned to normal or less than normal, except in the only animal of the nephritic group on which it was possible to make determinations after the blood pressure had fallen (rabbit 3031). In this case the response to all three pressor drugs remained elevated.

When hypertensive blood-pressure levels were regained by any means, the response to pressor drugs was again heightened. The duration of the pressor response to any of the drugs was not increased to the same extent as the height; there was no increased duration such as occurs with adrenaline following cocainization [Frolich & Loewi, 1910].

TABLE I. Pressor response to adrenaline before and after production of hypertension

Animal no.	Normal B.P. mm. Hg	Normal pressor response mm. Hg	Hypertensive B.P. mm. Hg	No. of days after 1st operation or serum injection	Pressor response during hypertension mm. Hg
2860 C	85 84-88	54, 56*	108	9	61*
2905 C	77 73-80	52	96 96 103 161 153	9 10 18 38 52	87, 84 70, 70, 70 89, 88 91, 91 42 (0.6 $\mu\text{g.}/\text{kg.}$) 63, 62, 62 (1.25 $\mu\text{g.}/\text{kg.}$) 46, 47 (0.6 $\mu\text{g.}/\text{kg.}$)
2907 C	78 75-80	23, 22	103	3	45
2908 C	95 87-97	46	108	3	78, 79
2921 C	105 102-108	57, 55	137 99	5 16	74, 70 46
3031 S	105 101-109	52, 50 (1.25 $\mu\text{g.}/\text{kg.}$)	136 113 101 104	4 23 44 67	117 (0.6 $\mu\text{g.}/\text{kg.}$) 150 (1.25 $\mu\text{g.}/\text{kg.}$) 48 (0.6 $\mu\text{g.}/\text{kg.}$) 65 (1.25 $\mu\text{g.}/\text{kg.}$) 60, 60 (1.25 $\mu\text{g.}/\text{kg.}$) 80, 80 (1.25 $\mu\text{g.}/\text{kg.}$)
3043 S	102 101-103	57 27 (1.25 $\mu\text{g.}/\text{kg.}$)	111 109	22 40	66, 68 42 (1.25 $\mu\text{g.}/\text{kg.}$) 36 (1.25 $\mu\text{g.}/\text{kg.}$)
3074 C†	79 73-83	43, 43 (1.25 $\mu\text{g.}/\text{kg.}$)	100	3	62, 64 (1.25 $\mu\text{g.}/\text{kg.}$)
3075 S	91 89-93	60, 61 28, 27 (1.25 $\mu\text{g.}/\text{kg.}$)	99 104 98	16 39 58	70, 76 39, 40 (1.25 $\mu\text{g.}/\text{kg.}$) 33 (1.25 $\mu\text{g.}/\text{kg.}$)
3124 S	74 72-79	34, 34 (1.25 $\mu\text{g.}/\text{kg.}$)	91 97	1 44	43, 39 (1.25 $\mu\text{g.}/\text{kg.}$) 59, 58, 57 (1.25 $\mu\text{g.}/\text{kg.}$)

C. Hypertension produced by constriction of renal artery.

S. Hypertension produced by injection of nephrotoxic serum.

* Dose 2.5 $\mu\text{g.}/\text{kg.}$ unless otherwise stated.

† Response determined in unanaesthetized animal.

TABLE II. Pressor response to tyramine before and after production of hypertension

Animal no.	Normal B.P. mm. Hg	Normal pressor response mm. Hg	Hypertensive B.P. mm. Hg	No. of days after 1st operation or serum injection	Pressor response during hypertension mm. Hg
2716 C	78	18*	142	13	60, 64*
	70-82		100	37	35
			83	123	15, 17
			91	164	24, 24
2737 C	102	42, 42	151	10	58
	98-100				
2798 C	92	56, 58	100	5	44, 46
	87-95				
2802 C	93	28, 30	124	6	56, 58
	88-96		98	42	24, 26
			159	47	56, 59, 61
2819 C	88	30, 33	121	10	52, 54
	85-92		130	42	82, 86
			113	133	57, 64
			145	180	113, 117
3031 S	105	52, 55	120	4	100
	101-109		104	67	79, 73
3043 S	102	16, 15, 16	105	24	18, 21
	101-103				
3074 C†	79	42, 45	104	4	66, 63
	73-83				
3075 S	91	15, 15	99	16	29, 32
	89-93		104	39	55
			98	59	31

C. Hypertension produced by constriction of the renal artery.

S. Hypertension produced by injection of nephrotoxic serum.

* Dose 0.5 mg./kg.

† Response determined in unanaesthetized animal.

The post-operative response of rabbit 2798 to tyramine was apparently an exception to the general rule. However, the blood pressure of this animal on the day of the experiment was only 5 mm. Hg above the highest normal reading, and laparotomy revealed that the clamp had slipped from the artery.

The post-operative response of rabbit 2797 to posterior pituitary extract was less than normal; death of the animal prevented confirmation of this observation. In all animals, once a hypertension had been established and an increased response obtained, the sensitivity to posterior pituitary extract was found to decrease steadily even though the blood pressure was still rising (e.g. rabbit 2905). This may have been due to a developing tolerance of the drug. If the drug was withheld for a long period (3 months), the response returned to its previous value (rabbit 2819).

TABLE III. Pressor response to posterior pituitary extract before and after production of hypertension

Animal no.	Normal B.P. mm. Hg	Normal pressor response mm. Hg			Hypertensive B.P. mm. Hg	No. of days after 1st operation or serum injection	Pressor response during hypertension mm. Hg		
		0.12 unit/kg.	0.06 unit/kg.	0.03 unit/kg.			0.12 unit/kg.	0.06 unit/kg.	0.03 unit/kg.
2716 C	78				136	9			
	70-82		36, 38		86	153		67, 70	
2797 C	111	26, 28			145	5	15		
	106-116								
2798 C	92	17, 17			108	4	29, 34		
	87-95								
2802 C	93		33, 35		147	4		64, 70	
	88-96				98	17		22, 21	
					165	22		46	26
					174	23		34	18
2819 C	88	35, 35			122	17	80	53	
	85-92				130	43		27, 29, 30	
					117	155		62, 63	
					124	167		75	56, 55
2905 C					158	207		98	45
	77				112	3		61	
	73-80				103	18		54, 56	
					130	25		52	
					143	40		47, 46, 44	
3031 S					150	75		36	
	105	47	25, 22		111	4		150	
	101-109				113	22		72	
					101	44			56
3043 S	102	43			111	22		61	
	101-103								
3074 C*	79		36, 36		100	5			70, 68
	73-83								
3075 S	91		15, 15		105	17			25, 26
	89-93				114	38			42
					98	59			21, 23
3124	74	14, 16			91	1		40, 43	
	72-79				97	30		22, 23	

C. Hypertension produced by constriction of the renal artery.

S. Hypertension produced by injection of nephrotoxic serum.

* Response determined in unanaesthetized animal.

During the course of the nembutal anaesthesia there may be a fall of blood pressure amounting to 30 mm. Hg. It was found that the response to any of the drugs used did not vary during the course of this fall, or during the recovery from it.

The response to acetylcholine was decreased in all animals following the appearance of hypertension (Table IV). As with the pressor drugs, there was no relation between (normal response)/(hypertensive response) and (normal B.P.)/(hypertensive B.P.). The experiments with rabbits 2905 and 3075 showed that there was a tendency for the sensitivity to return to normal over a period of time, though the original sensitivity was never regained. When the blood pressure returned to normal, the response reached its former value (rabbit 3124).

TABLE IV. Depressor response to acetylcholine before and after production of hypertension

Animal no.	Normal B.P. mm. Hg	Normal depressor response mm. Hg	Hypertensive B.P. mm. Hg	No. of days after 1st operation or serum injection	Depressor response during hypertension mm. Hg
		Dose			Dose
2860 C	85 84-88	I 24	108	9	I 18
		II 83			II 28
		III Pulseless			III 37
2905 C	77 73-80	I 14	96	9	I 13
		II 20			II 13
		III 31			III 16
			127	31	I 9
			130	35	II 16
			161	38	III 24, 23, 25
2907 C	78 75-80	I 20, 18	103	4	I 12
		II 31			II 17
		III 43			III 21
			145	75	III 24, 22
3075 S	91 89-93	I 41, 42, 44	105	17	I 11
					II 15
					III 19, 20
			107	65	I 38
					II 43
					III 48
3124 S	74 72-79	I 13	91	1	III 18
		II 19			I 13
		III 26			II 18
			92	2	III 19
					I 16
					II 21
			79	36	III 26

Dose I 2.5 $\mu\text{g./kg.}$, dose II 3.75 $\mu\text{g./kg.}$, dose III 5 $\mu\text{g./kg.}$

C. Hypertension produced by constriction of renal artery.

S. Hypertension produced by injection of nephrotoxic serum.

Effect of cocainization on hypertensive blood pressure. Experiments to determine the effect of cocainization according to the method of Tainter & Chang [1927] were carried out on four rabbits hypertensive as a result of constriction of the renal artery (rabbits 2716, 2819, 2905, 2921) and

on four nephritic rabbits (rabbits 3031, 3043, 3075, 3125). The blood pressure was followed for $\frac{1}{2}$ hr. before the injection of cocaine-HCl, and, after an interval of 15 min., for a period of 1 hr. afterwards. In no case was any significant change noted. These results indicate that the hypertension is not due solely to either adrenaline or tyramine.

DISCUSSION

It is impossible with the evidence presented to determine with certainty the cause of the changes in pressor and depressor responses demonstrated in the hypertensive animal. One can, however, eliminate as a cause an increased (or decreased) cardiac effect of the drugs used. This conclusion is supported by the results with posterior pituitary extract which owes its action solely to a peripheral vasoconstriction; the cardiac output is decreased [Tigerstedt & Airila, 1913]; and Holman & Page [1938] have shown that the cardiac output in the hypertensive animal is normal. Nor is it possible to explain the results presented by saying that they are due to a simple mechanical effect resulting from a decreased circumference of the arterioles. If this were so, one would expect the response to acetylcholine to be increased rather than decreased. Also, it was shown that during the period of anaesthesia the response does not depend on the level of the blood pressure at the moment of injection. With unanaesthetized animals, the response was found not to be influenced by fluctuations of blood pressure due to excitement. Working with smooth muscle from another source, viz. the bladder, Mellanby & Pratt [1939] found that in an isotonic preparation the response to adrenaline is not changed by varying the pressure over a wide range. These considerations eliminate such a simple explanation.

The similarity in the characteristics of the circulatory systems of the animals hypertensive as a result of constriction of the renal artery and the nephritic animals supports the conclusion that the mechanism of the hypertension is the same in the two cases.

SUMMARY

1. It has been found that the pressor response to adrenaline, tyramine and posterior pituitary extract is increased, and the depressor response to acetylcholine is decreased, in rabbits hypertensive as a result of constriction of the renal artery.

¹ 2. Similar results were obtained with rabbits hypertensive as a result of an induced glomerulonephritis.

3. Cocainization of hypertensive rabbits was found to have no influence on the blood pressure.

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THE ACTION OF ACETYLCHOLINE, ESERINE AND OTHER SUBSTANCES ON SOME MOTOR RESPONSES OF THE CENTRAL NERVOUS SYSTEM

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THERE is no clear indication of the significance of the presence of acetylcholine in the central nervous system. It is found in the grey matter [Stedman & Stedman, 1937, 1939] and in the same situation cholinesterase is present in strong concentration [Nachmansohn, 1939]. During perfusion of the brain with eserinated blood, acetylcholine is liberated from the brain, and the liberation is increased by KCl [Chute, Feldberg & Smyth, 1940]. It is claimed that the acetylcholine content of the cerebro-spinal fluid is increased by stimulation of the hypothalamus [Adam, McKail, Obrador & Wilson, 1938] and by stimulation of the central end of the vagus [Dikshit, 1934; Chang, Hsieh, Li & Lim, 1938], though negative results for central vagus stimulation have been reported [Feldberg & Schriever, 1936; Adam *et al.* 1938]. According to Schweitzer & Wright [1937*a, b*, 1938*a, b*] and Schweitzer, Stedman & Wright [1939], acetylcholine may influence central transmission processes by acting as an excitatory or inhibitory agent within the spinal cord. Apart from the observations on the knee-jerk by Schweitzer & Wright, information regarding the action of acetylcholine and of anticholinesterases on the central nervous system is meagre. Our experiments are concerned mainly with the action of acetylcholine and of eserine on central motor responses, particularly those to electrical stimulation of the cerebral motor cortex.

METHODS

The experiments were on cats. The cerebral motor cortex was stimulated by 50 c./sec. A.C. between 1 and 5 V. using the unit of Bayliss & Eggleton [1935]. Unipolar stimulation was employed; the stimulating electrode, which incorporated a light spiral spring, was maintained in

steady contact with the cortex by a holder which was screwed to the skull and served as the indifferent electrode. The head was fixed by a clamp and the cortex was kept warm and moist. The responses to stimulation were recorded by isometric spring myographs from the biceps in the forelimb or the tibialis anticus in the hindlimb, the limbs being immobilized by drills passed through the appropriate bones and fixed to uprights. In most experiments, stimulation was by short bursts of about 0.5 sec. at intervals of 5 or 10 sec. and the duration and interval were regulated by a Lewis contact-breaker. In a few experiments shorter (1 or 2 sec.) or longer (15 or 30 sec.) intervals were used. At intervals of 5 or 10 sec. the responses were remarkably steady and were little affected by cortical facilitation or extinction.

The pyramidal tract was stimulated by the same current and at the same rate. A hole was drilled in the base of the occipital bone immediately to one side of the middle line, the dura was opened to expose the pons, and a small needle concentric electrode was inserted into the lateral portion of the tract and securely fixed by a clamp. The responses were recorded from the tibialis anticus. From the same muscle the responses of the spinal flexor reflex were recorded; the ipsilateral tibial (popliteal) nerve was stimulated through a Collison fluid electrode, or a Sherrington glass-shielded electrode, by a.c. (less than 1 V.) or induction shocks or condenser discharges. When cortical response and the reflex were recorded simultaneously, the spinal cord was sectioned in the lower dorsal region. In many experiments, the opposite tibialis anticus was stimulated by a.c. through its motor nerve at the same rate as cortical or reflex excitation. Stimulation of other nerves, such as the central or peripheral ends of the vagus, was by faradic current.

Anaesthesia. The most satisfactory cortical responses were obtained under urethane (1.0 g./kg. intravenously) or dial (0.5 to 0.7 c.c./kg. intraperitoneally). A tube was tied in the trachea, and in most cases artificial respiration was given. For cortical responses chloralose, nembutal and nitrous oxide-oxygen mixture were found unsatisfactory. Very light ether anaesthesia was also tried; to prevent troublesome movements, the spinal cord was transected in the upper cervical region and artificial respiration was given. The responses of the orbicularis oculi to cortical stimulation were recorded. By this method motor impulses from the cortex, which did not traverse the spinal cord, were studied.

Injections and inhalations. Intracarotid injections were made in the following way. On the side to be stimulated the external carotid artery was divided between ligatures at 1 cm. distal to its origin, and the proxi-

mal segment was isolated by ligature and section of its branches. This segment was occluded by a narrow-bladed clip, and a blunted needle was tied in it and firmly fixed by a clamp. The needle was filled with the solution to be injected and the injections, which were retrograde, were made as rapidly as possible during momentary release of the clip. Injections of dye showed that the injected fluid passed immediately through the internal carotid artery into the cerebral vessels. Intravenous injections were made in the usual way.

To administer gas mixtures, such as carbon dioxide and oxygen, the tracheal tube was connected with light inspiratory and expiratory valves, the inspiratory valve leading to a spirometer containing the mixture; during control periods the animal breathed air through the valves.

Arterial blood pressure was recorded from the femoral artery. In many experiments the vagi were severed and the carotid sinuses denervated.

RESULTS

A. *Effects of acetylcholine on motor responses*

(1) *Intracarotid injection of acetylcholine.*

Injections of 0.001–0.1 mg. acetylcholine by themselves produced no contraction of limb muscles. The results were negative even when the background of cortical activity was enhanced by stimulating afferent fibres in the central end of a mixed nerve, such as the median, before and during the injection.

The same injections, however, had a very conspicuous action on the responses to electrical stimulation of the cortex. There was an immediate increase of the responses persisting for 10–30 sec., followed by a phase of depression, or even obliteration, of variable duration, after which the responses regained a normal level. The whole effect lasted usually between 1 and 5 min. Both the intensity and duration of the effect varied greatly in different animals; in some, a striking change was seen after 0.005 mg., in others 0.05 mg. or even 0.1 mg. were required for a similar effect. Usually the conspicuous phase after intracarotid injection was the initial increase of the responses (Fig. 1), and this was frequently associated with prolongation of the after-discharge. Initial increase was the only effect of small quantities of acetylcholine; depression appeared, and became more pronounced, as larger doses were injected. Control injections of saline, and of acid saline at the same pH as the acetylcholine solution, had no action, and the effect of acetylcholine was unaffected by denervating the carotid sinus or by section of the vagi. In most experiments repeated

injections of the same dose of acetylcholine produced very similar effects on each occasion, but in some experiments the variation was such that minor differences in the effects could not be regarded as significant.

Atropine had a strongly antagonistic action. Even a small dose abolished the depression (Fig. 1) and a dose of 1 mg./kg. prevented all changes, except the prolongation of after-discharge. The actions of acetylcholine and atropine on after-discharge were, however, very inconstant and were not specially studied.

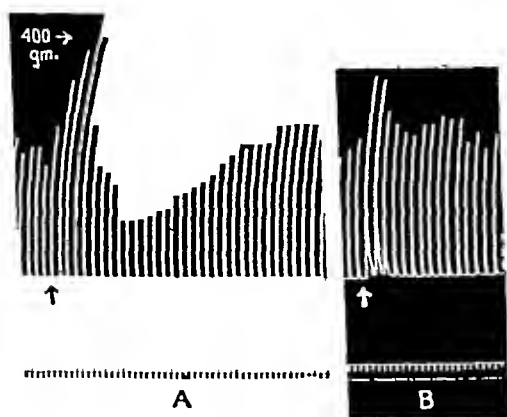


Fig. 1. Cat. Dial. Record: tibialis anticus responses to cortical stimulation. Time 10 sec. At arrows intracarotid injection 0.03 mg. acetylcholine. A, before atropine. B, 15 min. after 1.0 mg. atropine. Note antagonistic action of atropine.

The same changes appeared in the responses of the orbicularis oculi when the appropriate cortical area was stimulated under light ether and with the spinal cord sectioned in the upper cervical region. Large doses (0.1 mg.) were required to produce the change in these conditions.

Relation to respiratory changes. Intracarotid acetylcholine was always followed by pronounced respiratory changes, especially hyperpnoea. Since most experiments were done under artificial respiration and with the vagi cut, and since the hyperpnoea following acid saline injections was without effect it seemed unlikely that changes in pulmonary ventilation were responsible for the action on cortical responses. The action of carbon dioxide will be considered later.

Relation to circulatory changes. Intracarotid acetylcholine caused a sharp fall of blood pressure, and it seemed possible that the fall of pressure, presumably by altering the blood flow through the brain, was

responsible, in part at least, for the changes in cortical responses. According to Wolff [1929] acetylcholine dilates cerebral vessels, and it might be expected that immediately after intracarotid injection there would be an increased blood flow through the brain, followed by a diminution as the systemic blood pressure fell. Some features were clearly compatible with this view. The initial increase of responses corresponded roughly with the period before the fall of pressure, the depression with the period during and after the fall, and the cortical changes were readily suppressed by atropine, which would abolish the effects of injected acetylcholine on blood vessels. The possibility was further examined by a study of intravenous injection of acetylcholine and of other depressor agents.

(2) Intravenous injection of acetylcholine.

The effect on cortical responses of intravenous acetylcholine was very similar to that of intracarotid injection of the same doses (Fig. 2). Depression, sometimes extinction, of the responses was the chief feature; the initial excitatory phase was less obvious and was sometimes absent, but in other respects the similarity was very close. The effect was very readily suppressed by atropine, it was unaffected by vagal section, by denervation of carotid sinuses and by the substitution of artificial for natural respiration.

The site of action. In the doses employed, acetylcholine had no action on the responses of the muscles to stimulation of their motor nerves.

The spinal flexor reflex responses were considerably less sensitive to acetylcholine than the cortical responses. This was shown in two sets of experiments. In the first, with the spinal cord sectioned in the lower dorsal region, the responses to cortical stimulation were recorded from the biceps in the upper extremity, the flexor reflex from tibialis anticus (Fig. 3). In the second, with spinal cord intact, the tibialis anticus

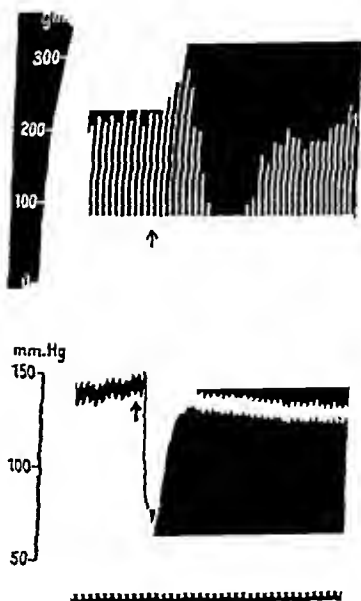


Fig. 2. Cat. Urethane. Vagi cut. Upper record: tibialis anticus responses to cortical stimulation. Lower record: blood pressure. Time 10 sec. At arrows intravenous injection of 5.0 μ g. acetylcholine.

was stimulated from the cortex and subsequently reflexly, using the same form and rate of stimulation. Doses of less than 0.1 mg. acetylcholine had little effect on the reflex. Any effect observed was a depression without initial increase.

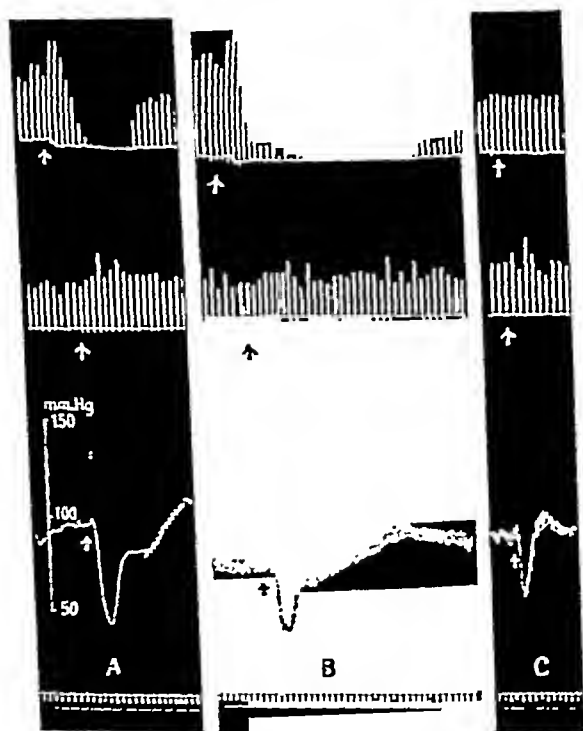


Fig. 3. Cat. Urethane. Vagi cut. Cord cut in mid-dorsal region. Upper record: biceps response to cortical stimulation. Middle record: tibialis anticus response to reflex stimulation. Lower record: blood pressure. Time 10 sec. A, before eserine. At arrow 10.0 μ g. acetylcholine i.v. B, after 0.125 mg. eserine. At arrow 1.0 μ g. acetylcholine i.v. C, after 1.0 mg. atropine. At arrow 100.0 μ g. acetylcholine i.v. Note acetylcholine effect on cortical responses potentiated by eserine and abolished by atropine. Reflex unaffected.

The pyramidal tract was stimulated and the action of acetylcholine was tested. In comparing the sensitivity of cortical, tract and reflex responses the order of stimulation was varied in different experiments to avoid fallacies arising from slight injury to the tract. Bearing in mind the doubtful significance of minor changes, we concluded that the tract responses were more readily affected than the reflex, but less readily than the cortex.

B. *The effects of certain depressor and pressor agents*

The following depressor substances were tested: other choline esters (doryl and mecholine), histamine, sodium nitrite and amyl nitrite. It was found that any of these substances injected in sufficient dosage to produce a steep fall of blood pressure affected the cortical responses in much the same way as did acetylcholine; in the case of choline esters, but only in them, the effects on blood pressure and cortical responses were prevented by previous atropinization. The correspondence between blood pressure fall and cortical changes was, however, by no means constant and exact, and the action of histamine and the nitrites was irregular.

Central vagus stimulation. The effect of central vagus stimulation on the knee-jerk has been studied by Schweitzer & Wright [1937a]. We found that its action on cortical responses and on the flexor reflex was very similar to that described by them for the knee-jerk. Occasionally, the depression of cortical responses was very prolonged, and during recovery the responses were irregular. The cortical responses were more susceptible than the reflex responses to central vagus stimulation, as well as to injected acetylcholine. The effects on blood pressure and motor responses of central vagus stimulation were only mildly antagonized by full doses of atropine. The action of eserine will be mentioned later.

Stimulation of the ipsilateral or contralateral cervical sympathetic trunk had no effect on motor responses.

Stimulation of the peripheral end of vagus. In some instances, depression of the cortical responses ensued, but this action was irregular. It did not appear after atropine.

The action of depressor agents, therefore, gave good reason to suspect that the changes produced by acetylcholine in cortical responses were, in part at least, a result of cerebral ischaemia. Accordingly we tested the effect of ischaemia by occluding the main arterial channels to the brain.

Cerebral ischaemia. All branches of the subclavian arteries, including the vertebrals, were tied in the neck and the right common carotid was ligated. During stimulation of the left cortex, the left carotid artery was suddenly occluded by a clip. The effect had a fair resemblance to that of acetylcholine (Fig. 4). It was noteworthy that obliteration of the responses persisted for some time after release of the clip.

Pressor agents

Adrenaline (0.05–0.5 mg.) generally had an effect similar to that of acetylcholine, usually depressant, sometimes excitatory, but unaltered by atropine.

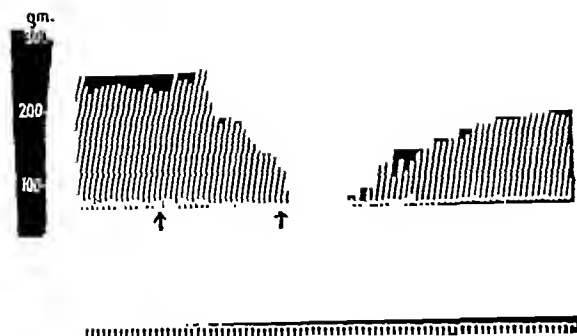


Fig. 4. Cat. Dial. Vagi cut. Carotid sinuses denervated. Vertebral arteries and right carotid artery tied. Record: tibialis anticus responses to stimulation of left cortex. Time 10 sec. Between arrows occlusion of left carotid artery. Note effect of cerebral ischaemia.

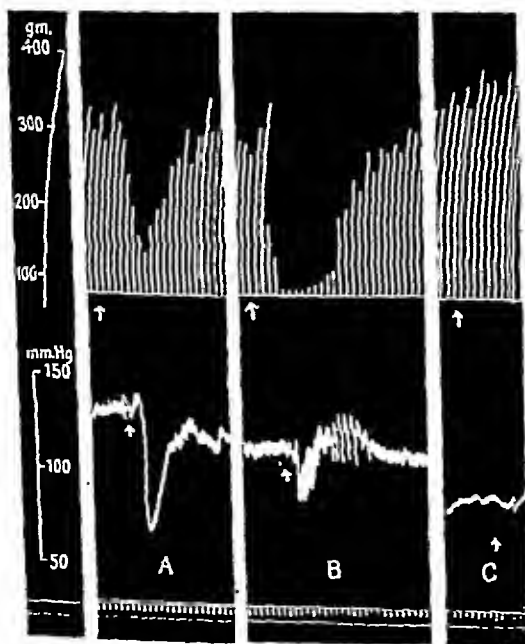


Fig. 5. Cat. Urethane. Upper record: tibialis anticus responses to cortical stimulation. Lower record: blood pressure. Time 10 sec. A, at arrows 5.0 μ g. acetylcholine, i.v. B, at arrows inhalation of 10% CO_2 in O_2 for 30 sec. C, at arrows inhalation of CO_2 after atropine 1.0 mg. per kg. Note effect of CO_2 on cortical responses abolished by atropine.

Carbon dioxide. Concentrations of 5% and upwards in oxygen produced a striking depression of the cortical responses (Figs. 5, 6); preliminary excitation was not observed. Usually a concentration of 10% in oxygen was necessary for a conspicuous action. High oxygen percentages by themselves were ineffective. The surprising feature was that the carbon-dioxide effect was readily suppressed by atropine, unless high

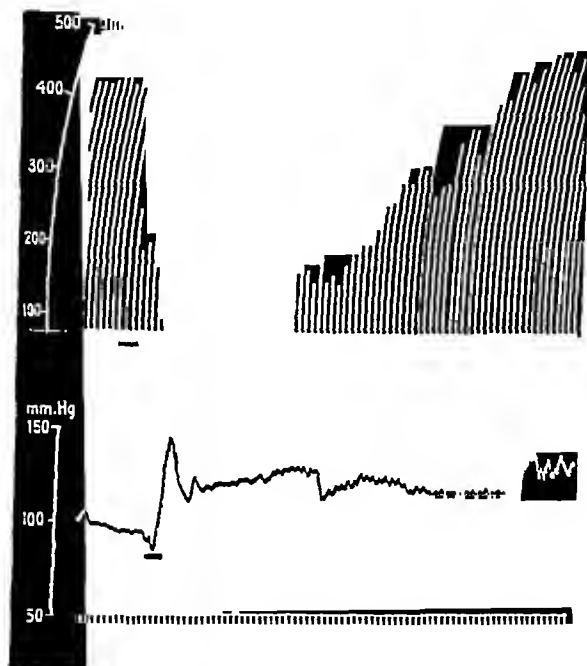


Fig. 8. Cat. Urethane. Vagi cut. Carotid sinuses denervated. Upper record: tibialis anticus responses to cortical stimulation. Lower record: blood pressure. Time 10 sec. At marks, 12% CO_2 in O_2 for 30 sec. Note depression of cortical responses by CO_2 associated with rise of blood pressure.

concentrations (30%) were used. Atropine did not affect the hyperpnoea and rise of blood pressure which accompanied carbon dioxide inhalation. Carbon dioxide had little effect on the flexor reflex, confirming the observation of Schweitzer & Wright [1937a] on the knee-jerk. Denervation of the carotid sinuses and section of the vagi did not alter the action of carbon dioxide on cortical responses.

Stimulation of afferent fibres. Faradic stimulation of the central end of the median nerve produced, in addition to a pressor effect, a very conspicuous increase of cortical responses without subsequent depression.

The increase appeared at once, and subsided in a few seconds when the stimulus was discontinued; it was unaffected by atropine.

Pituitary (posterior lobe) extract (0.5–1.0 c.c.) had no observable action on motor responses.

C. *The effects of eserine on motor responses*

(1) *On the responses to cortical stimulation.*

The usual effect of eserine (0.1–0.5 mg.) was a depression of the motor responses, commencing about 2–5 min. after injection. After a single dose of 0.5 mg., the responses were usually obliterated for periods as long as 45 min. and sometimes permanently. The depression was not merely a result of a general circulatory disturbance, because the smaller doses, up to 0.25 mg., had no effect on the blood pressure. Depression occurred also at slow stimulation rates (1 in 30 sec.). The depression was antagonized and sometimes prevented by a previous injection of atropine, but the antagonism was only partial, because once the responses were abolished, atropine, even in full doses, did not restore them, nor did it appear appreciably to hasten recovery. Depression was also observed in the responses of the face muscles to cortical stimulation under very light ether anaesthesia and after section of the cervical portion of the spinal cord. Surprisingly enough, reflex excitability in the head was simultaneously increased, as noted also by Chute *et al.* [1940] in perfusion of the brain.

A curious sequel of eserine was sometimes seen. After the eserine effects had disappeared, and the cortex was again responding to electrical stimulation, the responses could not be altered by acetylcholine, carbon dioxide or central vagus stimulation which, previous to eserine, had a pronounced action. The responses had apparently become "stabilized". The same condition was sometimes observed *during* the period of eserine action, when depression had been prevented by a small dose of atropine.

(2) *On the pyramidal tract responses.*

In five of six experiments eserine depressed or obliterated the tract responses. The depression was antagonized by atropine; it was not altered by excision of the cortex. In one instance the tract responses were increased by eserine. In all these experiments, the same doses of eserine obliterated the cortical responses.

(3) *On the flexor reflex.*

The usual effect of eserine was to increase the reflex responses. This agrees with the observations of Schweitzer & Wright [1937b] on the knee-jerk, but in our experiments the doses of eserine were small, the

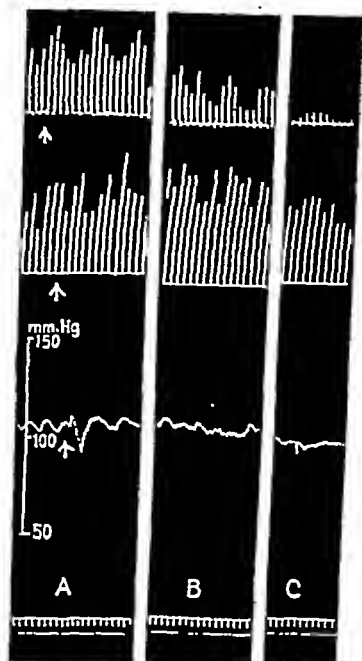


Fig. 7. Cat. Urethane. Cord cut in mid-dorsal region. Upper record: biceps response to cortical stimulation. Middle record: foot flexors response to reflex stimulation. Lower record: blood pressure. Time 10 sec. A, at arrow 0.25 mg. eserine i.v. B, after 5 min. C, after 30 min. Note depression of cortical responses by eserine; little change in reflex or blood pressure.

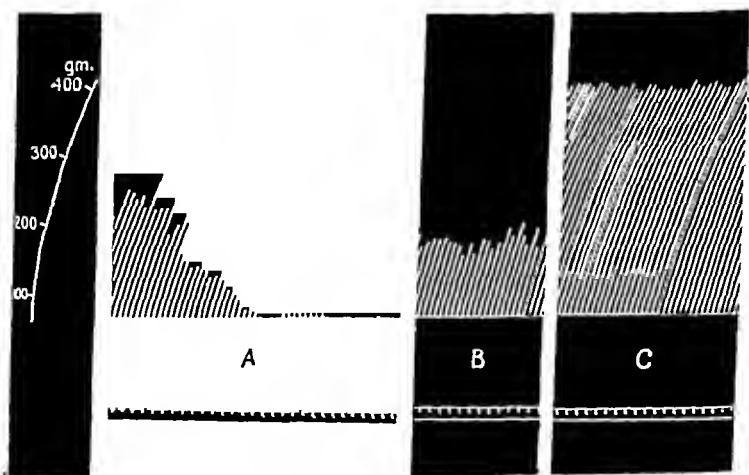


Fig. 8. Cat. Dial. Record: tibialis anticus responses. A, cortical, B and C reflex stimulation. Time 10 sec. A, 3 min. after 0.25 mg. eserine. Note onset of depression. B, before eserine. C, 12 min. after 0.25 mg. eserine. Note increased responses.

effects were not pronounced (Fig. 7), and were sometimes absent. On several occasions we demonstrated, in the same animal, obliteration of cortical and tract responses and enhancement of the spinal reflex (Figs. 8, 9).

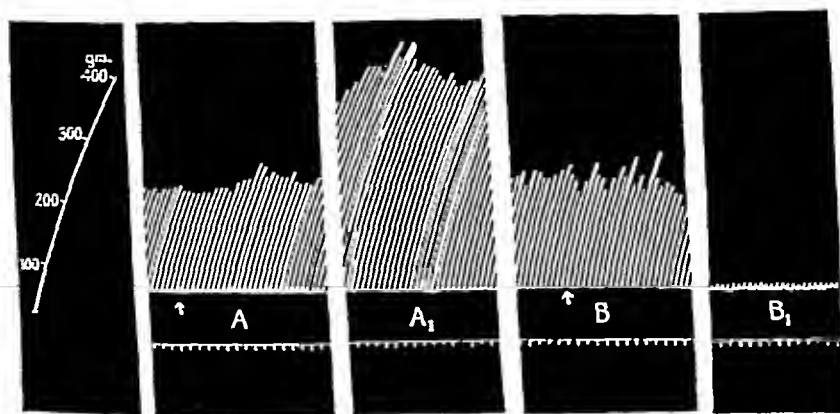


Fig. 9. Cat. Dial. Record: tibialis anticus responses. Time 10 sec. A, A₁, reflex stimulation. B, B₁, pyramidal tract stimulation. At arrows 0.25 mg. eserine. A₁, 12 min. after A; B₁, 12 min. after B. Note eserine increases reflex responses, depresses tract responses.

(4) On the neuro-muscular mechanism.

Except in a few experiments in which a slight increase of peripheral responses appeared, eserine had no action on the muscle stimulated through its motor nerve. It can be confidently asserted that the effects of eserine on cortical and tract responses, and probably also those on reflex responses, were central and not peripheral.

The central action of eserine was not altered by denervation of carotid sinuses or section of the vagi.

Eserine depression. Attempts to remove the depression caused by eserine were largely unsuccessful. The partial antagonism of atropine has been mentioned. If the responses were not completely obliterated, the height of the responses could be increased by afferent nerve stimulation, but only during the stimulation. KCl (0.1–0.5 mg.) occasionally produced a partial restoration, but the action was inconstant. Negative results were obtained with dextrose, sodium pyruvate, vitamin B₁, cortin and pituitary (posterior lobe) extract.

Eserine potentiation of acetylcholine action. Demonstration of a potentiating action of eserine on the effects of acetylcholine was rendered

very difficult by the depressant action of eserine on cortical response prevent severe depression, eserine was sometimes given in small repeated doses, or a small quantity of atropine was injected with the eserine. Under these conditions, the cortical responses not infrequently became resistant to the action of acetylcholine. In a few experiments, however, an undoubted potentiation of the acetylcholine effect was obtained (Fig. 3). In view of this difficulty of demonstrating potentiation importance could be attached to negative results, but positive results were probably significant. In testing the effect of eserine on central vagus stimulation some suggestion of potentiation was seen occasionally, the results were equivocal. In the case of carbon dioxide a positive result was obtained twice.

Prostigmine. The action of prostigmine on the cortical response was similar to that of eserine. Owing to its strong peripheral effects we did not make an extensive investigation of its central action.

D. Other actions on motor responses

(1) *Faster rates of stimulation.*

Brief periods of stimulation of the cortex at intervals of 1 or 2 seconds sometimes yielded regular responses. At these rates the responses were very sensitive to the action of acetylcholine, central vagus stimulation and carbon dioxide; even $1.0\mu\text{g.}$ or $0.5\mu\text{g.}$ acetylcholine produced obliteration. Unfortunately, the sensitivity passed off as the experiment proceeded, and comparison of effects on the responses became impossible.

(2) *Stimulation of the opposite motor cortex.*

A curious feature was that the cortical responses were inhibited by faradic stimulation of the opposite cortex but only during the stimulation. The effect had no resemblance to the action of acetylcholine.

DISCUSSION

The interpretation of these results must be approached with caution and for some of the observations we can do little more than record them.

Our inability to elicit motor responses by intracarotid injection of acetylcholine might be regarded as evidence against the view [Mille Stavraky & Woonton, 1940] that acetylcholine initiates or transmits impulses from the cerebral motor cortex. On the other hand, the evidence was not decisive, because the injection was not the "close range" type used for voluntary muscle by Brown, Dale & Feldberg [1936], and the response examined, contraction of muscle, was a somewhat gross index

ly. Using the more delicate test of the reflex, Forbes [1937] found that intracarotid injection of small doses increased the activity of the acoustic area of the cortex, as depressed or abolished it.

of injected acetylcholine on the motor responses to stimulation was striking. Whether injected through the carotid artery, the action was evidently exerted on the central nervous system and not on peripheral neuro-muscular mechanisms. To what extent the action was dependent on circulatory changes remained uncertain. It was suggested that the fall of blood pressure produced by acetylcholine when injected intravenously, was partly responsible for the depression in motor responses. Both the motor and vascular effects of acetylcholine and of other choline esters were suppressed by atropine, and a similar depression of motor responses was effected by a number of depressor agents and by cerebral ischaemia. Beecher and Forbes [1938] have demonstrated that a fall of blood pressure diminished the electrical activity of the cerebral cortex. There are, however, indications that the fall of blood pressure was not the only factor. By a suitable arrangement of dosage it could be shown that the motor responses elicited by injection of acetylcholine were enhanced by eserine and entirely abolished by atropine, and the pressure fall was practically equal in each case. The action of other depressor substances, such as histamine and amyl nitrite, on the motor responses was irregular and did not correspond closely to their effect on the electrical activity. Moreover, similar motor changes could be produced by substances such as adrenaline and carbon dioxide. In the case of carbon dioxide, which is also a powerful dilator of cerebral blood vessels (Munro, 1930), depression of cortical responses was associated with a decrease in cerebral blood flow and not a decrease of cerebral blood flow. It seemed, therefore, that injected acetylcholine modified the motor responses in an action which was in part independent of vascular changes. The action was not determined exactly, but apparently the cortical responses were more sensitive than the pyramidal tract responses and the reflexes were more sensitive than the flexor reflex. Presumably, acetylcholine acted on the cerebral cortex as well as on the spinal cord.

The effects of eserine were interesting though their significance is far from clear. The motor changes after the small doses used in these experiments were a result of central and not peripheral action. They could not be attributed to general circulatory disturbances nor, probably, to local changes, such as vasodilatation, in the central nervous system.

Eserine as a rule depressed the cortical and pyramidal tract responses increased the spinal reflex responses. In the spinal cord its action was inhibitory and excitatory. Schweitzer, Stedman & Wright [1939] concluded from a study of anticholinesterases that the tertiary bases, such as eserine, which penetrated the cell membrane, were excitants in the spinal cord, whereas quaternary bases, which exerted their action outside the cell-membrane, were depressants. We have shown, however, that anticholinesterase, eserine, had opposing actions at different levels in the central nervous system and even within the spinal cord. Merlis & Law [1939] found that the action of eserine on spinal reflexes varied with the reflex tested. The central action of eserine is evidently complicated and assumptions regarding the mechanism are at present scarcely permissible.

The effect of carbon dioxide on motor responses was an unexpected feature. The action was central and, since the spinal reflex was relatively insensitive, probably mainly on the cortex. The association of depression of cortical responses with an increased cerebral blood flow showed that depression was not necessarily indicative of cerebral ischaemia. Most surprising was the ready suppression of its motor effects by atropine and their potentiation by eserine. This gave strong presumptive evidence of the release of acetylcholine in the central nervous system when stimulated by carbon dioxide. Until more direct confirmatory evidence is forthcoming speculation as to the site and mechanism would be unjustifiable.

From collateral evidence there was more reason to expect evidence of release of acetylcholine by central vagus stimulation. As the motor changes were considerably resistant to atropine, the evidence of release depended upon demonstrating a potentiating effect of eserine. The results, though occasionally suggestive of potentiation, were neither clear-cut nor consistent. As already explained, these negative results under the experimental conditions were not specially significant.

It is clear that the further investigation of the central action of acetylcholine requires other experimental methods in which acetylcholine can be applied to the nerve cells with greater accuracy, and in which circulatory changes can be controlled. It should be emphasized that the conditions of our experiments did not provide a test of the possible function of acetylcholine as a quick transmitter of the effects of nerve impulses at central synapses; if the evidence did not support this conception, equally it did not forbid it. What was suggested, in the actions of injected acetylcholine, of eserine and of carbon dioxide, was

a slow or "muscarine-like" effect by acetylcholine which influenced the passage of motor impulses in the central nervous system. The nature of this process is very obscure and more information is obviously desirable.

SUMMARY

1. Intracarotid injection of acetylcholine (0.001-0.1 mg.) produced a striking alteration of the responses to electrical stimulation of the cerebral motor cortex of the cat. The responses were at first increased, and later depressed. Excitation was the main feature. The action was on the central nervous system. It was strongly antagonized by atropine.

2. Intravenous injection of the same doses of acetylcholine had similar effects, but depression was the prominent feature. The action was again central and was partly on the cerebral cortex and partly on the spinal cord. It was easily suppressed by atropine, and was potentiated by eserine.

3. Similar changes in motor responses were produced by various depressor agents and by cerebral ischaemia, but also by some pressor substances. Probably the central action of injected acetylcholine was in part independent of vascular changes.

4. Small quantities (0.1-0.5 mg.) of eserine usually depressed or obliterated the responses to stimulation of the cortex and of the pyramidal tract, and simultaneously excited the spinal flexor reflex. The action was central, and probably was independent of vascular changes.

5. Eserine acted on the cerebral cortex and also on the spinal cord. In the spinal cord its action was both inhibitory and excitatory.

6. Carbon dioxide depressed the cortical responses by a central action which was antagonized by atropine and potentiated by eserine. This was presumptive evidence of the release of acetylcholine in the central nervous system under stimulation by carbon dioxide.

7. There was no clear indication of the release of acetylcholine by central vagus stimulation.

8. Our experiments provided no information regarding the possible function of acetylcholine as a quick transmitter at central synapses.

9. There was a definite suggestion of a slow action of acetylcholine, which influenced the passage of motor impulses in the central nervous system.

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A PHYSIOLOGICAL STUDY OF THE SKIN RESISTANCE RESPONSE IN MAN

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THE present study is an attempt to investigate further the mechanisms underlying the psychogalvanic response described by Ferri (1888). This response is the reduction in resistance of the skin to the passage of a low-voltage direct current following various sensory stimuli. No attempt has been made to analyse the causes of the skin R.M.R. or Tarchanoff reaction. Vigoroux (1888) stated that the changes in resistance were due to variations in the size of the small skin vessels. Veraguth (1906), however, ascribed this response to activity of sweat glands, as he found that it could still be obtained after devascularization of the limb. More recently Aveling & McDowall (1925) came to the conclusion that the vascular change played the main part, whereas Darrow (1929) insisted that the response arose from glandular activity. The subject matter pertaining to this response has been well reviewed by Landis (1932). Owing to the divergence of opinion as to the cause of the response, it was felt that further investigations might be carried out, and it is the purpose of this paper to record and discuss the results.

METHOD

The apparatus used to record changes in the skin resistance consisted essentially of a modified Wheatstone bridge and galvanometer of the d'Arsonval type with a period of 2 sec. Calibration of the skin resistance was effected either by a suitable increase in one of the ratio arms or by the addition of 100 to 500 Ω in the variable arm of the bridge. Skin electrodes were made of small zinc plates coated with a layer of cotton wool heavily impregnated with zinc sulphate kaolin paste. They were applied to the dorsal and ventral aspects of the distal phalanges, the

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mately 2500Ω in subjects accustomed to the procedure. Like resistance changes were obtained from the whole surface of the thorax and abdominal wall and from the four extremities so long as the subject was adequately warmed. Otherwise it was impossible to obtain the response above the wrists and ankles. The resistance response was also recorded from the scaphae of the ears. Examination of the ear for sweat showed that it occurred only along the antihelix to a slight degree after the subject had been well warmed, and even then was not obtainable in all subjects. Further, the sweat response to faradism at this site was lacking. Vaso-motor responses, on the other hand, have been recorded from the ear in man with a plethysmograph [Golla, 1921].

Effect of skin temperature on latency of response. It was observed that the latencies of the response from a single area were much longer at the commencement of an experiment than later in its course. This was particularly true for the toe. For instance, in one test the latencies from the third left toe varied from 2.5 to 3.04 sec. in the early records; later times of 2.6-2.8 sec. were recorded. The great variability appeared to be related to the fact that initially the subject was not as warm as later in the experiment. It was decided to study the effects of local cooling upon the latency of the response.

The subject was brought to a state of maximal vaso-dilatation by warming so that like skin temperatures were recorded on similar fingers or toes on both sides. Under such conditions at similar loci on the two sides the recorded onsets of the resistance responses to faradic stimuli were found to be synchronous or to differ only by 0.01 sec. One hand was then plunged into cold water until chilled to $12^{\circ}\text{C}.$, while care was taken to avoid cooling taking place in the other hand by the continued immersion of the legs in water at $45^{\circ}\text{C}.$ Simultaneous recordings of the resistance response were then made from the fingers of both hands, the temperatures of which were read at each response. The results in two experiments are shown in the following graph (Fig. 1). Against the difference in temperature is plotted the time difference of the latencies of the response in similar fingers of each hand. It will be seen that as the temperature of the cooled finger approaches that of the constantly warm finger, the difference of their latencies diminishes. It is of importance, therefore, in comparing the latencies of skin resistance response at separate areas to ascertain that the temperature is the same in both areas.

Latency of response in different skin areas. The latency of the response following a break shock from a secondary coil was found to vary according

nail surface being avoided, or at a distance apart of 2 cm. where other areas of the body were selected. They were freshly made for each experiment. With this precaution this type of electrode was found to be most satisfactory in that polarization was minimal.

The subject sat or lay in a separate, partially darkened room. A state of maximal peripheral vasodilatation was maintained by immersion of one or more limbs in water at 40–45° C., by radiant heat and by exclusion of air currents. The temperature of the skin area at the electrode site was taken with thermojunctions. The stimuli used were deep breaths, break-shocks from a secondary coil applied at diverse sites, and unexpected noise of fairly constant intensity and pitch.

Various procedures were utilized in modifying the local conditions. Devascularization was effected by rolling a rubber bandage up the finger from the tip to the base, a tourniquet being applied before removal of the bandage. Rapid occlusion of the circulation was obtained by application of a tourniquet to the base of the finger, or by rapid inflation of a sphygmomanometer cuff applied to the arm. Localized atropinization was effected by iontophoresis with a 3% solution of atropine sulphate, the maximum duration being 10 min. with a constant current of 3 mA.

Plethysmographic records of vaso-motor responses were made from a control finger and also from the tip of the finger used for devascularization and atropinization. Recording tambours of maximum sensitivity were used for this purpose. The presence of sweating was searched for after painting with an iodine-alcohol solution and spraying with starch, or tested by prolonged faradization of the skin area. Failure to detect the black stippling was taken to indicate absence of sweating.

RESULTS

By these methods it was found that with careful preparation and application of the electrodes the total skin resistance of the distal phalanx varied from 7500 to 25,000 Ω in different subjects. Since such a wide range of variation was obtained no attempt was made to compare values of resistance observed in different experiments.

Following a break-shock from a secondary coil, noise, or a deep breath, a diminution in resistance took place. This was in agreement with the findings of Golla [1921] and of others. For quantitative comparison of these resistance changes in the same subject the stimuli were not repeated too frequently. Because of the relative constancy of the stimulus received, the faradic shock was found most useful. In the finger the diminution of resistance following such stimuli amounted to approxi-

in the finger with occluded circulation, a change in skin resistance which occurred at the same time as the decrease in volume of that finger. This change was synchronous with a vaso-constriction observed in the control finger of the same hand. The sweat response to local faradism was obtainable from the finger with occluded circulation 5 min. after application of the tourniquet. This experiment indicated that simple occlusion of the blood flow did not diminish the skin-resistance response. In keeping were the observations that vaso-constriction and sweating remained during the period of the test.

A study of the effect of occlusion of the circulation on the latent period was also undertaken. It was found that simple occlusion of the circulation did not alter the latency of the response, provided that cooling of the occluded limb was avoided. The times of onset of the response were compared in similar fingers of the two limbs. The arm due to have the circulation occluded was kept immersed in water at 36.5°C . save for the fingers which were exposed to radiant heat. After occlusion of the circulation in this arm by means of inflation of a sphygmomanometer cuff, the difference in time of onset of the response from the two limbs, when temperatures were the same, was of the same order as before the occlusion.

Effect of devascularization on the skin resistance response. When the resistance response was taken from a finger devascularized by an Esmarch's band and tourniquet, an immediate reduction in the size of the response was uniformly found. In the early responses following devascularization the decrease amounted to about 75-80% of the response in the same finger originally. This result was obtained with all types of stimuli, although in a number of cases the reduction in the response following a deep breath was more marked than the reduction in the response to painful stimuli. With a prolonged period of devascularization there was associated decrease in the degree of the response and after intervals of 10 min., or more in some subjects, the response could not be elicited. It was noted that abolition of the response was coincidental with disappearance of local sweating produced by faradism.

The temperature of the devascularized limb was maintained at the normal level with difficulty. When it was uncontrolled, studies showed that, although the initial decrease in the response occurs at the time of maximum fall of temperature in the conditions of the experiment, the progressive decrease in the response was not associated with further temperature changes. However, when cooling of the devascularized part was prevented, the decrease in the response still occurred.

to the part from which the response was recorded. For instance, the shortest latency was obtained in the case of the response from the skin over the upper thoracic vertebrae. The next shortest latency was from the front of the chest, while those of responses from the ear, finger and toe followed in that order. The location of the painful stimulus was found to make no difference in the value of the absolute latency in any

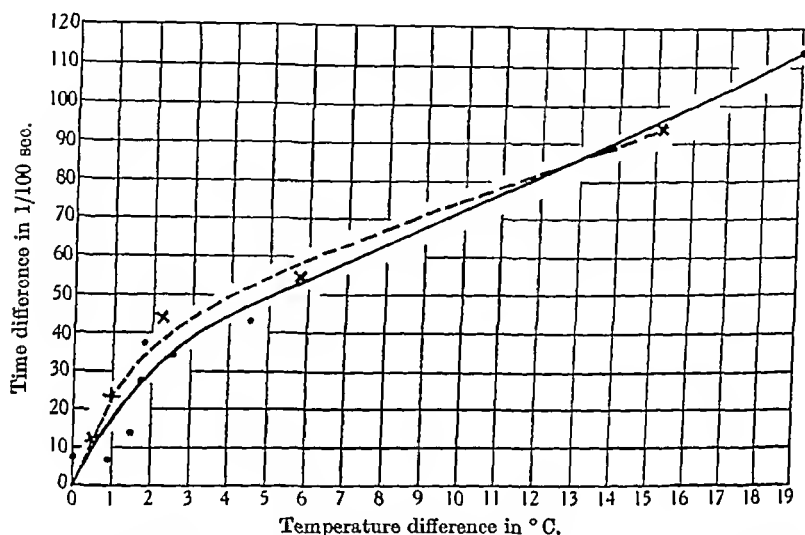


Fig. 1. Physiological study of skin resistance response. Time difference plotted against temperature difference. \times = Subject A; \bullet = Subject B. In both subjects one finger was kept warm with vessels in a dilated state; the other finger was cooled. The points represent the time difference of onset of reaction following stimulus plotted against the temperature difference of the two fingers.

case. The significance of the variation of the latency of response in different areas will be discussed elsewhere [Carmichael, Honeyman, Kolb & Stewart, 1941].

Effect of arterial occlusion on the skin resistance response. In order to determine whether arterial occlusion affected the response a comparison was made of the responses before and after interrupting the circulation either by occlusion of the brachial artery with a sphygmomanometer cuff or by application of a tourniquet around the base of the finger. The magnitude of the response immediately after interruption of the circulation was of the same order as before. An experiment was performed in which the circulation of one finger was occluded and a plethysmographic record made of the volume of its distal part while the resistance electrodes were applied to the same area. After a deep breath there was,

the hand and then from the foot, but rarely elsewhere. His subjects were not warmed before examination. The present work shows that, in suitable conditions, the response may be obtained from most body surfaces. In a total of ninety-three experiments on twenty healthy young adults there was no failure to detect the response over the large area of body surface examined. In this series only one individual failed to give the response from the ear.

Reports on the latency of the response have varied considerably. Golla [1921] found the latency for the finger to be 2 sec. Jung [1939] more recently gives 1.7 sec. as a typical value. Insufficient attention has been paid to the general state of the subject in regard to vaso-dilatation and sweating, as well as to the local temperature conditions. Gildemeister [1923] noted that while the latent period might vary with the temperature of the skin, it is fairly constant at a fixed temperature. It is insufficient, however, to control the temperature of the electrodes. Optimal conditions for eliciting the response must be ensured by warming the subject and promoting vaso-dilatation and sweating. In such conditions it has been shown that the latency of the response varies according to the area of the body from which it is recorded. In the determination of the relation between local temperature and latency of the response an attempt was made to maintain the subject in the same steady state of vaso-dilatation, but inasmuch as the local cooling necessarily disturbed this by reason of sensory stimuli and fall in venous blood temperature, ideal conditions could not be maintained.

The degree and the latency of the skin-resistance response are uninfluenced by cessation of blood flow induced by rapid arterial occlusion, if care be taken to maintain the temperature and sweating at its previous level. It has been shown that after cessation of blood flow, vaso-motor and sweating activities are not impaired for a short period. Analysis of these factors has been attempted previously with variable results. Veraguth [1906] and Jung [1939] exsanguinated the arm and reported the response as unaltered. Densham & Wells [1927] stated that they found a 50% decrease in the response following this procedure, while Goadby & Goadby [1936] found that the skin-resistance response disappeared while the E.M.F. change remained unaffected. It has been possible to show that on devascularization the response is initially reduced by 75% with a further progressive decrease in value towards abolition, which is related to the cessation of sweating. The time interval is variable, but the response is generally not detectable after 10 min. From these experiments it may be said that the response is not wholly

Release of the circulation was not followed by an immediate recovery of the response, and a time interval of varying length was necessary for the development of a full response.

Under isothermic conditions a study was made of the effect of devascularization on the latency of the response. It was found that, immediately following devascularization, there was no significant difference in the time of onset of the response in the devascularized and control fingers.

In all the above experiments the completeness of devascularization and the absence of vaso-motor activity were controlled by plethysmographic records from the tip of the devascularized finger.

Effect of atropinization. The presence of the response having been demonstrated after cessation of blood flow and of vaso-motor activity, the effect of the abolition of sweating was investigated. The response was consistently found to persist after atropinization of the skin area by iontophoresis. There was no tendency towards a progressive decrease in the value of the response, nor was the response ever wholly abolished.

Atropinization was found to have no appreciable effect on the skin temperature of the finger used. Further, the latencies in the atropinized finger did not differ from those of the control finger.

Absence of sweating in the finger subjected to iontophoresis was demonstrated in each subject by faradism, whilst sweating could still be obtained from adjacent fingers of the same hand or from the opposite hand. Vaso-constrictions were demonstrated in the atropinized finger.

Combination of atropinization and rapid occlusion of the circulation by means of a sphygmomanometer cuff applied to the arm gave results which varied from a marked diminution of the response to complete abolition. This variation in response occurred even in the same individual in different experiments. It is not easily explicable except in terms of tonicity and responsiveness of the vessel walls.

DISCUSSION

In studies of the skin resistance and of the reflex diminution in resistance that follows sensory stimuli, the maintenance of proper conditions of sweating and maximal peripheral vaso-dilatation, as indicated by skin temperature, becomes of first importance. This has been amply demonstrated by the effect of cold on both the distribution and latency of the response.

According to Gildemeister [1923], who examined the entire surface of the body in man, the skin-resistance response is obtained best from

SUMMARY

A study of the physiological basis of changes in skin resistance following various sensory stimuli has been made in man.

If the subject has been adequately warmed the response may be obtained from any part of the extremities, the body wall and the ears.

The latency of the response varies according to the part of the body from which it is recorded and also with the temperature of that part.

The response is indicative of activity of the sympathetic nervous system, being dependent upon the synchronous development of both vaso-constriction and of sweating, occurring when either of these components is present, but being abolished when both are absent.

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vascular in origin, although the proportion due to sweating cannot be definitely assigned, because of some coincidental reduction.

Darrow [1929] has criticized the vaso-motor explanation on the grounds first that the skin resistance response may occur often without a vaso-constriction, secondly that vaso-constriction may not necessarily be simultaneous with the response and that recovery of the resistance value to the previous level may be delayed beyond the vaso-motor return. In studying Darrow's records it is to be seen that his recording system must have been of low sensitivity, for no vaso-constriction was obtained with a deep breath. Further, the exact time relations of vaso-constriction and of the skin resistance response cannot be directly compared because of the different degrees of inertia in the two methods of registration. Further evidence against sweating as the sole cause of the response is its presence in a case of anhidrosis due to absence of sweat glands verified histologically, as reported by Gilchrist [1927]. Waller [1919], however, has shown that the reflex can be obtained after administration of atropine. The validity of his results has been demonstrated although a reduction in the value of the response takes place after localized atropinization by means of iontophoresis. This indicates the part played by vaso-motor activity in the production of the reflex. It is of interest to record that there is no difference under isothermic conditions between the latencies not only of the atropinized and the control finger, but also of the devascularized and of its corresponding control finger. In both cases the control fingers were in a state of maximal vaso-dilatation. It follows, therefore, that the time relations of the reflex response of vaso-constriction and of sweating following upon a sensory stimulus are identical. This of necessity implies that each have nervous mechanisms which are essentially similar in the sum of the time characteristics of their central pathways, ganglionic relays and peripheral pathways.

The dependence of the skin resistance response on the activity of the sympathetic nervous system is illustrated experimentally by its abolition with a combination of the effects of atropinization and devascularization. It is also shown clinically in cases of damage to the system. In one case with complete bilateral lumbar sympathectomy no response was obtained from the toe, while in another case with a lesion of the cauda equina involving motor and sensory roots from L_2 downwards good responses were obtained.

It may, therefore, be concluded that the change in skin resistance to a given stimulus results from activity of the sympathetic nervous system and is dependent upon two factors—sweating and vaso-motor activity.

METHOD

The subject was placed in a darkened room, extraneous stimuli being kept at a minimum. A steady state of maximal peripheral vasodilatation was maintained by the immersion of one limb in hot water at 45° C., and by radiant heat. A constant current was applied to the skin at the site selected through zinc plate electrodes covered with zinc sulphate and kaolin paste and rigidly fixed about 2 cm. apart. To record changes in the skin resistance, modified Wheatstone bridges were used with d'Arsonval galvanometers worked at the maximum sensitivity practicable. Their period was 2.0 sec. and intrinsic latency from a steady base 0.02 sec. Records were taken on fast-moving bromide paper. In a given experiment the stimuli used were constant break-shocks from a secondary coil and were applied to a point removed from the dermatome corresponding to the sympathetic outflow concerned in the response. After each stimulus readings of the skin temperature at the electrodes were taken with thermojunctions. Between each stimulus there was a sufficient interval of time for the skin resistance to return to its previous steady level.

RESULTS

If the subject is in a state of vaso-dilatation and the skin at the site of the electrodes kept suitably warm, a skin resistance response may be recorded from most areas of the body. The thoracic dermatomes appeared to offer a suitable area for determining conduction velocity, since it is probable that the post-ganglionic fibres for each dermatome spring from the same sympathetic ganglion. Pairs of electrodes were placed on the back and front of the chest in the distribution of the fifth thoracic dermatome and the differences in the time of onset of the responses under isothermic conditions determined in a number of subjects. Thus in subject S. W. the distance between the electrodes was 0.43 m. The response from electrodes placed anteriorly on the chest occurred 0.21 ± 0.04 sec. later than that from the electrodes on the back (Fig. 1). This gave a conduction velocity in the postganglionic fibres of 2.05 m./sec. Results from other subjects are shown in Table I and range from 2.05 to 2.3 m./sec. Records were also made to determine whether velocities in the proximal and distal parts of the nerves were equal. In a typical test the electrodes were first placed on the back and front of the chest in the fifth dermatome at a distance of 0.40 m. The latency of the onset of the response was 0.205 sec. greater for the anterior chest than for the posterior. The velocity was thus 1.90 m./sec. The anterior electrodes

PERIPHERAL CONDUCTION RATE IN THE
SYMPATHETIC NERVOUS SYSTEM OF MANBY E. A. CARMICHAEL, W. M. HONEYMAN, L. C. KOLB
AND W. K. STEWART*From the Research Unit, National Hospital, Queen Square, London**(Received 30 September 1940)*

IN view of the lack of data on the velocity of conduction in the peripheral autonomic system of man, the following investigation was undertaken. During experiments on the nature of the skin resistance [Carmichael, Honeyman, Kolb & Stewart, 1941] it was noted that there was a marked constant difference in the latency of the responses in the toe and in the finger. With the disclosure of a greater variety of sites from which the response was elicitable in suitable circumstances, it appeared that a method was here available for the determination of the conduction rate of the sympathetic system. The response of diminution in the skin resistance is due to a vaso-motor effect as well as an altered activity of the sweat glands. Both of these effects are due to sympathetic stimulation, and it seemed that for two skin areas with their sympathetic supply from the same ganglionic station the difference in the latencies of the responses must be accounted for by the greater nerve length to the area more remote from the station.

The method consisted in determining the time difference in the onset of the response at selected points following the stimulation of the skin by the break shock from a secondary coil. This time difference was taken to correspond to the difference in length of the peripheral nerve pathways involved. Since the areas of skin should be supplied by the same sympathetic outflow from the cord, responses were simultaneously recorded from the proximal and distal parts of the leg and arm, from areas at the back and front of the chest in the same dermatome. Responses from the ear and finger were also recorded. Observations on healthy young adults were made from these various sites and repeated on different days.

METHOD

The subject was placed in a darkened room, extraneous stimuli being kept at a minimum. A steady state of maximal peripheral vasodilatation was maintained by the immersion of one limb in hot water at 45° C., and by radiant heat. A constant current was applied to the skin at the site selected through zinc plate electrodes covered with zinc sulphate and kaolin paste and rigidly fixed about 2 cm. apart. To record changes in the skin resistance, modified Wheatstone bridges were used with d'Arsonval galvanometers worked at the maximum sensitivity practicable. Their period was 2.0 sec. and intrinsic latency from a steady base 0.02 sec. Records were taken on fast-moving bromide paper. In a given experiment the stimuli used were constant break-shocks from a secondary coil and were applied to a point removed from the dermatome corresponding to the sympathetic outflow concerned in the response. After each stimulus readings of the skin temperature at the electrodes were taken with thermojunctions. Between each stimulus there was a sufficient interval of time for the skin resistance to return to its previous steady level.

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for velocity approximate closely to those obtained from the responses in the rib area, and are likewise near the corrected velocities from the ear and finger. Observations were also made from the deltoid and from the elbow and finger. The calculated velocities showed no significant differences.

The velocity in the leg was similarly determined, the electrodes being placed at the buttock or above the knee and over a toe. There are certain disadvantages in these sites. The localization of the outflow from the cord of the sympathetic fibres to these parts in man, the site of their ganglionic relay and its distance from the cord are all unknown. There is, however, considerable uniformity in the results obtained from a number of subjects. The rate in the leg is considerably less than that in the other parts of the body, ranging from 0.85 to 1.27 m./sec. for the distance between knee and toe in seven subjects. The velocities for the proximal and distal halves of the leg were also checked against the velocity of conduction in the entire leg length. In one subject the gluteus-knee velocity was 0.86 m./sec., the knee-toe 0.87 m./sec. and the gluteus-toe velocity 0.91 m./sec.

DISCUSSION

The skin-resistance response in any part depends on the activity of the sympathetic nervous system. As has been discussed previously, there are two factors in this diminution of skin resistance, activity of the sweat glands and vaso-constriction. In the limits of accuracy possible by our methods in man there has been found no appreciable difference in the latency of the response in any part of the body when either of these elements, vaso-constriction or sweating, is eliminated. This means that, whatever the spinal afferents, and central reflex paths for vaso-constriction and sweating for one area, the effective time characteristics *in toto* are essentially similar. Nevertheless it might be suggested that the latencies of skin-resistance response would not yet be comparable in different sites, where patently the factors of vaso-constriction and sweating are quantitatively different. Thus in the finger tip with its great capacity for changes in blood flow, the factor of vaso-constriction is considerably greater than in the skin of the proximal parts of the limb. Indeed, total latencies of the responses in different sites may be analysed for peripheral nerve velocity only if the effector latencies are equal. In the absence of knowledge as to the stimulation parameters of vessels and sweat glands in different sites and likewise as to the temporal relation of their activity to the reduction in skin resistance, the assumption is made that under isothermic conditions any differences in their latencies are not of a

were then moved to the axilla, a distance of 0.26 m. now intervening. The time difference was found to be 0.14 sec., giving a velocity of 1.89 m./sec., practically the same figure as obtained for the greater length of nerve.

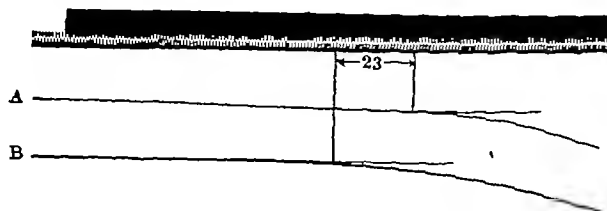


Fig. 1. Record taken from anterior chest wall (A) and from posterior chest wall (B). Break-shock at commencement of dark area and above time marker in 1/100th sec.

TABLE I. Conduction velocity of sympathetic fibres as determined from a number of sites in different subjects

Subject	...	S. W.	S. T.	H. O.	F. A.	L. I.	W. O.
Velocity in m./sec.							
Chest		2.05	2.30	2.10	2.05	2.10	—
Ear-finger		2.17	2.18	2.02	2.17	2.23	—
Shoulder-finger		1.80	2.08	1.93	1.50	2.17	1.83
Leg		1.27	1.00	0.96	1.09	—	0.85

Calculations of velocity were also made from the differences in the time of the responses in the ear and the finger. The total length of sympathetic nerve to the finger was reckoned as the distance from the 7th cervical vertebra which overlies the appropriate thoracic outflow. In a typical subject it was 0.23 m. for the ear and 0.96 m. for the finger. It is not, however, legitimate to reckon the time difference of the onset of the response at these two sites as representing the time taken in traversing the additional length of 0.73 m. The result of 2.17 m./sec. so derived fails to take into account the disparity in the lengths of the preganglionic fibres which amount to about 10 cm., since the ganglion station for the ear is probably in the superior cervical ganglion, and that of the index finger probably in the stellate. If preganglionic conduction velocity in man is comparable to that in animals, then it is possible that a velocity of 12 m./sec. may have to be allowed for the preganglionic fibres. Accordingly, the results from finger-ear difference should be diminished by approximately 8%.

Results based on time differences of the resistance response from the finger and from an area over the deltoid are also given in Table I. The appropriate ganglion cell stations for the fibres to both these areas are assumed to be in the stellate ganglion. The correction required in the case of the ear is, therefore, not necessary. It is seen that the figures

cervical ganglion in the cat, Eccles [1935] found in four cases rates of conduction in the S_2 fibres varying from 1.7 to 5 m./sec. Emphasis must be placed on the fact that the skin resistance response is the effector response of summed impulses travelling in a number of different fibres with probably a relatively large temporal dispersion. It may be that failure to record such high velocities as found for the onset of the C elevation in the cat depends on the conditions of summation determined by the plexal endings of the sympathetic fibres on the blood vessels.

The observation that the rate of conduction of impulses in the hind limbs is less than that in the arms is not easy of interpretation. It may be noted that Langley [1896] found large grey fibres to be rarer in the lower lumbar region than higher in the sympathetic chain.

The fact that there is no measurable decrease in speed of conduction towards the periphery is in agreement with the findings [Gasser & Erlanger, 1937] that there is a linear relationship between the nerve distance and the time taken by the components of the A elevation in the frog's sciatic. Marshall & Gerard [1933] demonstrated a uniform decline in conduction rate towards the periphery, but only for the fastest fibres. This is in agreement with the analysis of Hatai [1910], who found that the rate of increment of internodal length and the decrease in diameter towards the periphery was much greater in the larger fibres than in the smaller medullated and non-medullated fibres.

SUMMARY

In man the velocity of sympathetic nerves was determined by means of the skin resistance response.

The velocity of conduction in the postganglionic sympathetic nerves to the skin was found to vary from 2.17 to 1.80 m./sec. in the upper extremity, from 2.30 to 2.03 m./sec. in the chest, and from 1.27 to 0.85 m./sec. in the leg.

The limitations of the method are indicated.

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significant order. Then, if in any two parts with similar central connections latencies of the response to the same stimulus are compared, will be seen that all the components of the absolute latency are common with the exception of the times for the peripheral pathway. Effect latencies assumed equal, the time difference in the latencies thus represents the delay of impulses in traversing the greater length of postganglionic nerve to the more peripheral portion of the skin surface dermatome under investigation.

The range of variation of the time difference between the latencies of the responses from any two parts was found not to be greater than $\pm 6\%$ for isothermic conditions. This was true for stimuli from a variety of sites. The range of conduction velocities estimated varied considerably for different subjects and for different areas. For the chest and upper extremity, values from 1.5 to 2.3 m./sec. were obtained and for the lower extremity from 0.85 to 1.27 m./sec. In general it may be stated that for each subject there is a close similarity between the conduction velocities in the chest, where one dermatome is used, and in the upper extremity where different dermatomes, but of the same sympathetic outflow, are used.

The figures derived from the difference in the latencies of the responses in the ear and finger are necessarily subject to the condition that the characteristics of the superior cervical and stellate ganglia are similar. A correction is made for the greater preganglionic pathway in the case of the ear. This correction amounts to -8% if the conduction rate in the preganglionics be 12 m./sec.—the velocity in the preganglionic myelinated fibres of the S_2 cells in the superior cervical ganglion of the cat [Eccles, 1935]. Some support for the tentative assumption of this figure for conduction velocity comes from the statement of Bishop & Heinbecker [1932] that the preganglionic conduction rates in *Macacus rhesus* correspond to those of the cat. It may be remarked that at higher preganglionic velocities this correction is only slightly increased, e.g. at 20 m./sec. it would be -11% . With the correction of -8% the range of velocities from the ear-finger observations lies between 1.84 and 2.05 m./sec., thus remaining within the range of velocities derived from the chest and arm experiments.

Gasser & Erlanger [1937] analysed the contribution of the grey rami communicantes to the action potential of the sciatic nerve in the bull frog. Stimulation of these grey rami produced a double C elevation, C_1 travelling at 0.7 m./sec. and C_2 at 0.44 m./sec. In the like but more difficult preparation in the cat and dog, typical values for C were about 1 m./sec. and 1.6 m./sec. Using the postganglionics of the superior

preparation "perabrodil" was used in place of the diodrast employed by the American workers. The inulin concentration in plasma and urine was estimated by the diphenylamine method [Herbert & Davison, 1938], and perabrodil by a modified Kendall method [1920]. Blood volume was determined by the vital red method [Keith, Rowntree & Geraghty, 1915], and blood urea by urease and nesslerization [Archer & Robb, 1925].

RESULTS

(1) Normals

Inulin clearance was determined in eight normal subjects, and perabrodil clearance in six. The results have been corrected to conform to an "ideal" surface area of 1.73 sq. m., and are given in Table I. The mean

TABLE I. Inulin and perabrodil clearance in normal subjects

	No. of 15 min. periods	Mean inulin clearance c.c./min.	Mean perabrodil clearance c.c./min.	Inulin perabrodil clearance ratio %
N 1	3	118	—	—
N 2	4	106	—	—
N 3	3	118	630	19
N 4	4	115	532	22
N 5	4	133	607	22
N 6	3	117	437	27
N 7	3	148	520	28
N 8	4	102	496	20
Mean of series		120	537	23

of the normal inulin clearances was 120 c.c./min., and the range 102–148; the normal perabrodil clearances averaged 537 c.c./min., with a range of 437–630. These inulin clearances accord well with Smith's series of twenty-five normals (mean 122.5 c.c./min., range 100–153). Our values for perabrodil clearance are below the average of 820 c.c./min. given by Smith's series of eight subjects, with diodrast; it may be noted, however, that Chesley, Connell, Chesley, Katz & Glissen [1940], in twenty-one normals, found a renal blood flow usually between 700 and 900 c.c./min. corresponding to a diodrast clearance of 350–500 c.c./min.

(2) Patients with alimentary haemorrhage

Notes on the patients. P 1. Age 45. ♂. Duodenal ulcer. On admission, moderate shock, pulse rate 100, blood pressure 95/50; blood urea 100 mg. %, Hb 58 %, haematocrit 30 %, blood volume 4.1 l. After blood transfusion (850 c.c.), Hb 64 %, haematocrit 30 %, blood volume 5.4 l. Plasma inulin range 108–155 mg. %, organic iodine range 1.42–1.63 mg. %.

P 2. Age 32. ♂. Gastric erosions. On admission, pulse rate 84, blood pressure 118/84; blood urea 96 mg. %, Hb 56 %, haematocrit 23 %, blood volume 4.9 l. After blood transfusion (1200 c.c.), blood urea 42 mg. %, Hb 65 %, haematocrit 28 %, blood volume 5.8 l. Plasma inulin range 86–105 mg. %, organic iodine range 0.59–1.53 mg. %.

INULIN AND PERABRODIL CLEARANCE AFTER ALIMENTARY HAEMORRHAGE IN MAN

BY D. A. K. BLACK,¹ J. F. POWELL AND A. FORD SMITH

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(Received 4 October 1940)

THE work of Smith and his collaborators [1937, 1938, 1939] has shown that the clearance of inulin provides a satisfactory measure of glomerular filtration in man, while the clearance of diodrast approximates to the effective flow of plasma through the kidney, so that effective renal blood flow may be calculated from the diodrast clearance and the haematocrit ratio. Although these methods have been frequently applied to the study of organic renal disease, they have been little used in patients with alkalosis, diabetic coma, and persistent vomiting or haemorrhage, where retention of nitrogenous end-products indicates disorganized renal function in the absence of primary structural changes in the kidney. McCance & Widdowson [1939] have, however, used inulin clearance in an investigation on the renal failure of diabetic coma; the small amounts of inulin cleared indicated a very considerable diminution in glomerular filtration. It appeared desirable to determine whether diminished inulin clearance was present in "extrarenal azotaemia" of different origin; and at the same time to find out if there was any relation between diminution of total blood volume and the changes in glomerular filtration and effective renal blood flow. Patients with severe alimentary haemorrhage were chosen for this purpose, since previous studies have shown that diminution of blood volume, rise of blood urea, and depression of the urea clearance are common findings in these patients [Black, 1939; Black & Leese, 1940].

METHOD

The method for inulin and perabrodil clearance was essentially that of Smith, Goldring & Chasis [1938]. It was found that a more constant plasma inulin level could be obtained by using 12 g. of inulin in the priming infusion, in place of 15 g.; and a 35 % solution of the Bayer

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TABLE IV. Inulin and perabrodil clearances—patient 3

	A. On admission		B. 4 weeks later (No transfusion given)	
	Inulin	Perabrodil	Inulin	Perabrodil
Period 1	103	243	131	495
Period 2	58	138	104	435
Period 3	28	83	102	396
Period 4	143	650	141	575
Period 5	103	386	—	—
Mean	86	300	120	475
Mean (corrected for area)	86	300	120	475
Effective renal blood flow		420		750
Fraction of plasma filtered (%)		28		25

genous absorption from the large amounts of blood in the bowel. The first and third patients had their initial clearance estimation during the period when the blood urea was rising, while in the second patient, admitted a day after the haemorrhage, the urea concentration had already begun to fall.

(a) *The inulin clearance.* During the period when the blood urea was high or rising, the inulin clearance was depressed, in one patient to 23 c.c./min., and in the other to 86 c.c./min. In both these patients, the blood volume was reduced by more than a litre. In the patient with the greater reduction in inulin clearance, the general appearances of shock were more pronounced, and the blood pressure was lower; on the other hand, the pulse rate was equally rapid in both patients, while the blood volume was actually lower in the patient who showed less depression of inulin clearance. When the blood volume in the first patient was raised by transfusion, the inulin clearance increased from 23 c.c./min. to 55 c.c./min. (see Fig. 1); but factors other than the increase in blood volume cannot be completely excluded, since during transfusion the patient's condition improved in other respects, the blood pressure rising, and the signs of shock disappearing. It would appear that a diminished glomerular filtration rate is not exclusively related to a low blood volume or blood pressure, or to the intensity of the clinical syndrome of shock.

Two inulin clearances were measured in the second patient at a time when the blood urea had already begun to fall, and when the blood volume was little, if at all, reduced. The clearance at this time was found to be much higher than in a determination made after full recovery; the amount of glomerular filtrate was thus increased during the period when urea was being rapidly excreted. This finding might be associated with an

P 3. Age 51. ♂. Gastric erosions. On admission, moderate shock, pulse rate blood pressure 140/90; blood urea 70 mg. %, Hb 66 %, haematocrit 28 %, blood volume 2.6 l. After recovery, without transfusion, blood volume 5.0 l. Plasma inulin range 123 mg. %, organic iodine range 1.12-1.42 mg. %.

In the first two patients, inulin and parabrodil clearance was estimated on admission, after transfusion, and after recovery (see Tables II and III). In the third patient, who did not require transfusion, the clearances were determined on admission, and after recovery (Table IV).

TABLE II. Inulin and parabrodil clearances—patient 1.
(All the values are expressed in c.o./min.)

	A. On admission		B. 17 hr. later (after transfusion— 850 c.c.)		C. 3 months later	
	Inulin	Parabrodil	Inulin	Parabrodil	Inulin	Parabrodil
Period 1	10	23.5	34	77	79	365
Period 2	6	18	70	128	75	396
Period 3	48	97	52	109	81	428
Period 4	20	43	42	104	87	457
Mean	21	45	50	105	80	412
Mean (corrected for area)	23	50	55	116	89	453
Effective renal blood flow	71		166		810	
Fraction of plasma filtered (%)	46		48		20	

TABLE III. Inulin and parabrodil clearances—patient 2

	A. On admission		B. 20 hr. later (after transfusion— 1200 c.c.)		C. 5 months later
	Inulin	Parabrodil	Inulin	Parabrodil	Inulin
Period 1	183	480	170	358	99
Period 2	89	270	160	372	140
Period 3	139	430	245	470	134
Period 4	294	1140	142	278	140
Mean	201	575	179	369	128
Mean (corrected for area)	177	505	158	325	112
Effective renal blood flow	660		450		
Fraction of plasma filtered (%)	35		49		

After severe haemorrhage into the stomach or duodenum, the blood urea rises within a few hours to 70 mg. % or more; on the following day as a rule, it falls to a value only slightly above normal, and remains at this level for some days, before falling completely to normal. The available evidence suggests that the early rapid rise of blood urea is related to the fall in blood pressure and blood volume after the haemorrhage, while the persistent slight elevation of blood urea is due to nitr

observed either in normal subjects or in the same patients after recovery. We were inclined to ascribe these fluctuations to the difficulty of collecting accurate samples of urine in the presence of oliguria; but McCance [1940] has found similar irregularity in the inulin clearance of infants, and believes that it represents an actual variation in the amount of glomerular filtration from one period to the next. Since the bladder was well washed out during each period, the fluctuations observed by us may also indicate true changes in the rate of glomerular filtration. This suggests that the distortion of renal function after haemorrhage may include a reversion to a more primitive state of intermittent glomerular action such as occurs in human infants and in Amphibia.

(b) *The perabrodil clearance.* In the two patients showing reduction of inulin clearance at the time of haemorrhage, the clearance of perabrodil was also reduced, and to an even greater degree. In the first patient the inulin/perabrodil clearance ratio was 46 and 48 % in the two estimations done before and after transfusion, while after full recovery the ratio had fallen to 20 %. In the third patient, who showed a smaller degree of depression in inulin clearance, the inulin/perabrodil clearance ratio was 28 % at the time of haemorrhage, and 25 % after recovery. The second patient, who had a very high inulin clearance, had a perabrodil clearance well within the normal range. The inulin/perabrodil clearance ratio was 35 % before, and 49 % after transfusion.

It is difficult to assess the precise significance which should be attached to perabrodil clearance in patients with poorly functioning kidneys. Criticism of the identification of perabrodil clearance with effective renal plasma flow has not been lacking, even as regards normal subjects; and since the complete removal of perabrodil from the plasma depends on the integrity of tubular action, it seems likely that with damaged kidneys the perabrodil clearance may fall short of the actual effective plasma flow; it is hardly probable, for instance, that the renal plasma flow during the first clearance estimation on the first patient was as low as 50 c.c./min. In these circumstances, the ratio

$$\frac{(\text{perabrodil clearance})}{(\text{plasma percentage})} \times 100$$

represents only an approximation to the renal blood flow; and the inulin-perabrodil clearance ratio gives a high estimate of the fraction of plasma which is filtered. With these reservations, there remains evidence to suggest a very considerable reduction in renal blood flow during the period of rising blood urea, while during recovery the renal blood flow returns to normal. During the period of reduced renal blood flow the

increase in the number of functioning glomeruli. Richards and Schmidt have shown that in amphibia the number of functioning glomeruli is quite variable; but Winton [1937] is not satisfied that a similar variation in the number of functioning glomeruli plays any significant role in the mammalian kidney. Moreover, in a subject whose normal inulin clearance was 137 c.c./min., we found that the clearance was only 145 c.c./min. after the blood-urea level had been raised to 60 mg. % by the ingestion of 30 g. of urea. Another possible explanation of high inulin clearance in

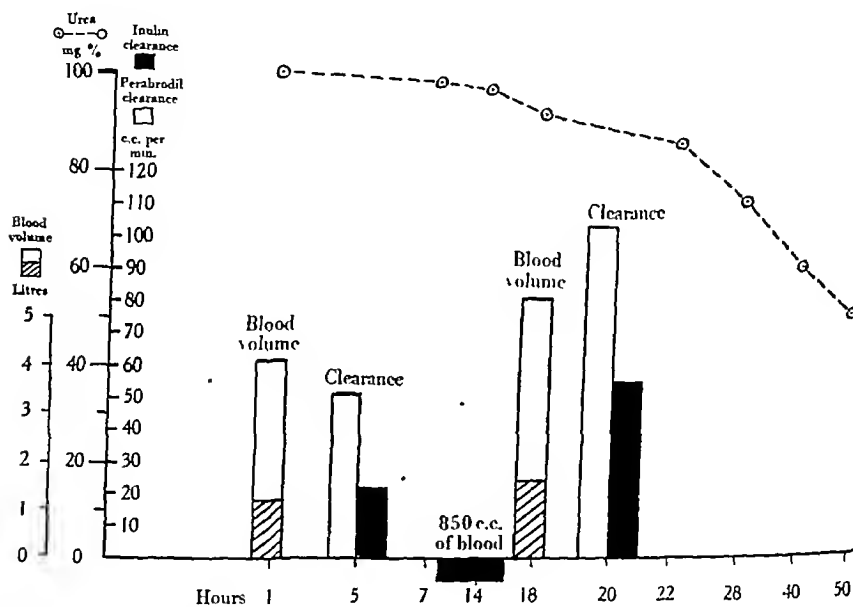


Fig. 1. Changes in blood volume and blood urea, and in inulin and perabrodil clearance, after transfusion in P. 1. The lower, hatched portion of the blood volume column indicates total red cell volume.

the second patient may be found in the low haematocrit value (23 %); although the effective renal blood flow was only 660 c.c./min., 505 c.c. of this were plasma, and it may be that filtration is favoured by a high plasma/cell ratio in the blood, provided that the blood volume is normal. In harmony with this hypothesis are some observations on a woman with aplastic anaemia, in whom an increase in haemoglobin concentration, due to repeated transfusion, from 30 to 82 %, was attended by a fall in inulin clearance from 71 to 59 c.c./min.

In all the estimations made at the time of haemorrhage, the clearances showed a much greater variation from one period to the next than was

concentration is rising, and normal or high during the period of recovery from the azotaemia.

(b) There is no close relation between the fall in glomerular filtration rate and the blood volume or blood pressure.

(c) When inulin clearance is diminished, perabrodil clearance is also reduced, and to an even greater extent, so that the inulin/perabrodil clearance ratio, which approximates to the fraction of plasma actually filtered, is raised.

(d) Fluctuation in the clearances from one period to the next was present at the time of haemorrhage, and disappeared after recovery.

2. The functional renal impairment in patients with haematemesis is more closely related to a fall in renal plasma flow than to any other single factor.

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inulin/perabrodil clearance ratio is high, as compared with the average value of 23 % in six normal subjects; this suggests that partial compensation for the reduced flow of plasma through the glomeruli may be effected by an increase in the proportion of plasma filtered.

DISCUSSION

Nature of the renal impairment. It has already been noted that the changes in glomerular filtration do not bear any constant relation to blood volume, to blood pressure, or to the degree of anaemia. There is, however, some degree of parallelism between the inulin and perabrodil clearances. If one assumes that the perabrodil clearance represents the renal plasma flow with accuracy, the inulin clearance is less depressed than the diminution in renal plasma flow would lead one to expect; if, however, as seems possible on the grounds earlier stated, perabrodil is not completely cleared by kidneys with distorted function, the true plasma flow would be significantly higher than the perabrodil clearance, and its diminution would correspond more closely to the depression of inulin clearance. Direct determination of renal blood flow in the explanted kidney, by Van Slyke, Rhoads, Hiller & Alving [1934], has shown that changes in renal blood flow are attended by similar fluctuations in the clearance of urea. Although the parallelism between glomerular filtration rate and renal blood flow is much less complete in our cases, the discrepancies may be due only to anomalous excretion of perabrodil by the abnormal kidney; and the relation between glomerular filtration rate and estimated plasma flow is much closer than that between inulin clearance and blood volume or blood pressure. The observed deficiency in glomerular filtration is thus best explained as the consequence of a diminished supply of plasma to the filtering surface. The reduced plasma flow to the kidney is determined in turn by diminished blood volume and blood pressure, and by disturbance of the circulation by shock; since these primary causes of reduced plasma flow are acting together, and in varying degree in different patients, no one of them bears a constant relation to the amount by which the glomerular filtrate is diminished.

SUMMARY

1. Inulin and perabrodil clearances have been measured in normal subjects, and in three patients with severe haemorrhage into the digestive tract.

(a) The inulin clearance is considerably reduced while the blood urea

The rabbit was chosen as the first experimental animal because it is relatively large and because modifications of its activity are well known and well defined. The abdomen was opened under aseptic conditions and small stitches of 36 s.w.g. silver wire were sewn at short intervals along the mesometrial border of one horn of the uterus from the vaginal end to the junction with the uterine tube. The stitches were inserted in the mesometrium about 2 mm. from the uterus to avoid direct irritation of that organ, and in the spaces between the blood vessels to minimize interference with the blood supply. From five to eight



Fig. 1. Positive from rabbit X 6 (actual size) to show the appearance of the stitches.

stitches were used and each was tied in a distinctive fashion so that the order could be recorded and recognized later in the photographs (see Fig. 1). In two experiments there was an adhesion between one of the stitches and the bladder, but no adhesion to intestine was ever found. No reaction round the stitches was seen at post-mortem examination even when the stitches had been in position for several months; the absence of inflammatory changes was confirmed by histological examination in all cases. Inflammatory changes have been shown by Laufer & Reynolds [1938] to produce uterine motility.

THE MOVEMENTS OF THE UNLOADED UTERUS

By G. H. BELL

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It is well known that the activity of the smooth muscle of the intestine is greatly modified by tension within the lumen, but the effect of tension on the muscle of the uterus has not received so much attention. It has been shown by Newton [1933] that the activity of the pregnant guinea-pig uterus *in vitro* is considerably modified by altering the load on the recording lever, and also that the threshold dose of oxytocin varies with the loading. It would not be wise, however, to apply these findings to the behaviour of the uterus *in vivo*; direct comparison of the activity of the uterus of the pregnant guinea-pig *in vivo* and *in vitro* has shown that the behaviour of the same piece of uterus is quite different under the two conditions (Bell, unpublished). As far as is known, no method of recording the uterine contractions *in vivo* has been described in which the uterus is subject neither to tension nor to distension. The pressure used in many experiments, especially in the human experiments, to distend the uterine balloon is very high indeed. An accidental finding is described by Allen & Reynolds [1935] thus: "In the course of an experiment the intrauterine balloon... became bulged, and increased in capacity... the contractions became much greater in this case while in the other experiments of this series the uteri became quiescent." Reynolds [1937], reviewing the subject, said: "Further consideration ought to be given, in the future, to the characteristics which various procedures impart to the kymographic records of uterine activity." The experiments which will be described were designed to eliminate tension and to investigate the movements of the uterus under conditions as far as possible normal.

METHODS

The method, briefly, was to take serial X-ray photographs of wire stitches placed alongside the uterus. (Compare Barcroft, Harris, Orahovats & Weiss [1925] on the movements of the spleen.)

smooth curve of this contraction strongly suggests that the error of measurement is, in fact, quite small. Not very much stress can be laid on the smaller alterations of the curve—e.g. from 4 to 10 min. in this case.

There were some struggling movements of the animal at 11 min. but the contraction started at 10 min. It has frequently been noted in other

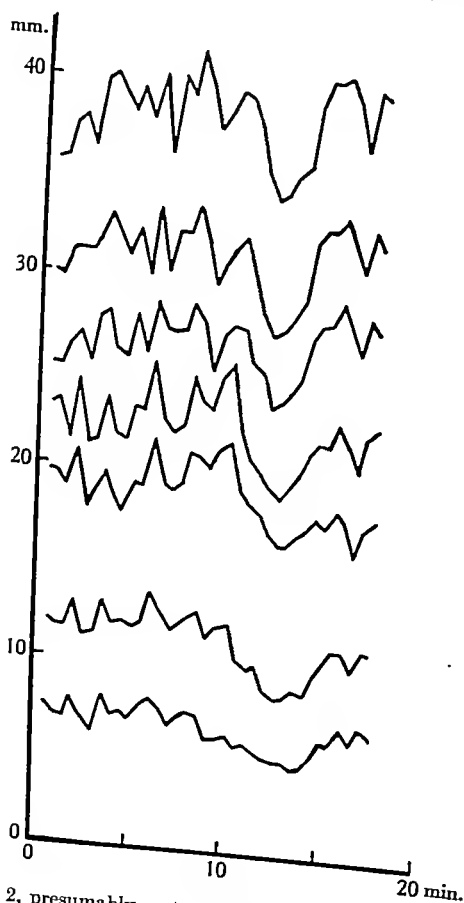


Fig. 2. Rabbit X 2, presumably oestrous, 2 weeks after insertion of silver stitches.

experiments where uterine movements were being recorded directly on a drum that a uterine contraction may produce restlessness even in an anaesthetized animal. When, as in the present case, the animal is conscious, struggling might be expected more frequently. Since contractions are associated fairly often with struggles, but a struggle is not necessarily accompanied by a contraction, it is probable that the uterine movement

paring the lengths at maximum relaxation). Injection of 0.02 unit of pitocin was followed by a contraction which may have been spontaneous.

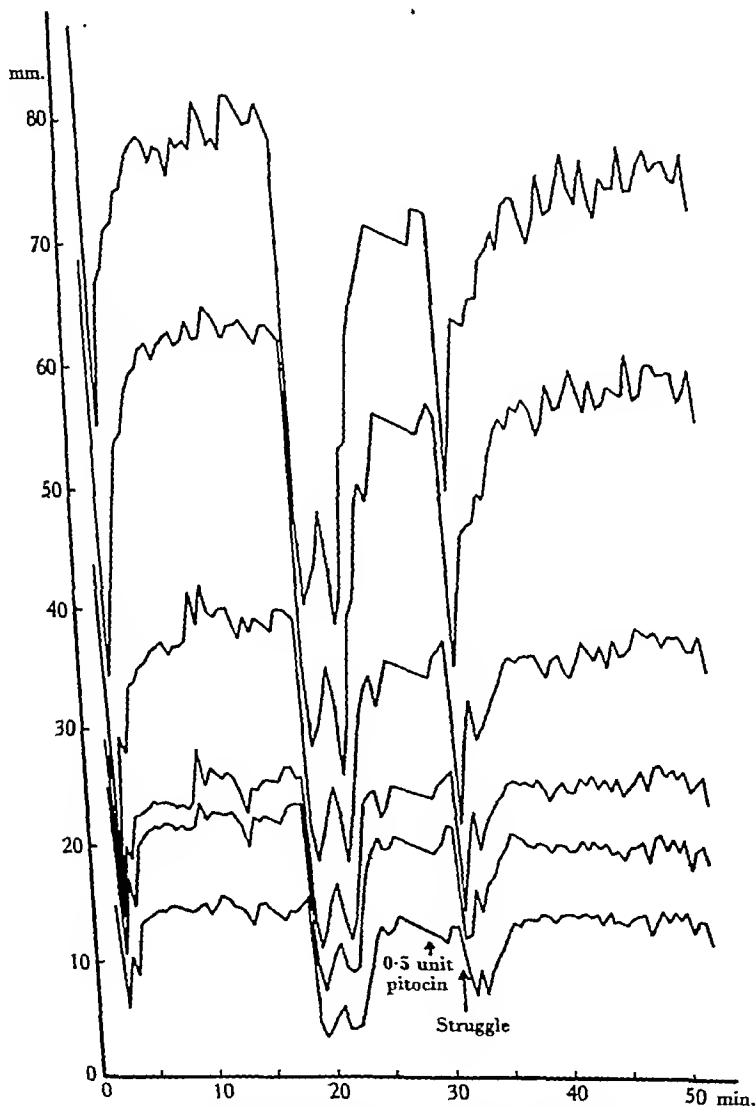


Fig. 4. Rabbit X 4. Stitches inserted 1 April 1937. Mare pituitary powder injected 17 July 1937. Experiment on 26 July 1937.

This dose would almost certainly have produced a marked effect had the usual methods of recording been used.

is the primary occurrence. It is difficult to imagine that any of the uterine movements illustrated here were painful—that is, if human experience is of any value as an *analogy*.

Rabbit X 3 was spayed and silver stitches were inserted along the left uterine horn; 1 week later photographs were taken. The curve (left-hand of Fig. 3) shows a preliminary relaxation followed by a very slight contraction. The early relaxation is of the same extent as the contraction

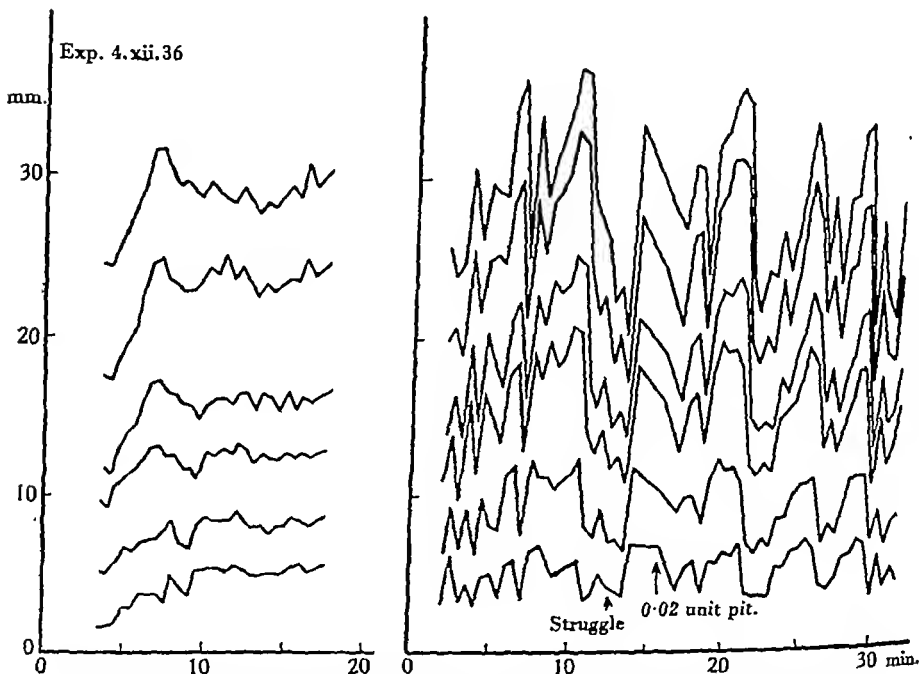


Fig. 3. Rabbit X 3 spayed 27 November 1936. Left-hand graph obtained 4 December 1936. Then injected with oestrone 4-11 December 1936. Right-hand graph obtained on 12 December 1936.

seen in Fig. 2, but generally speaking the uterus is less motile. A small piece of uterus taken from the right horn showed histologically the typical spayed appearance. An adhesion between the vaginal stitch and the wall of the bladder was broken down. Beginning on the next day subcutaneous injections of 0.01 mg. oestrone in olive oil were given twice daily for 1 week (total dose 0.14 mg.). On the day after the last injection another series of photographs yielded the second graph of Fig. 3. The difference between the two graphs is striking: the oestrin treatment has increased the movements of the uterus and has increased the total length (com-

paring the lengths at maximum relaxation). Injection of 0.02 unit of pitocin was followed by a contraction which may have been spontaneous.

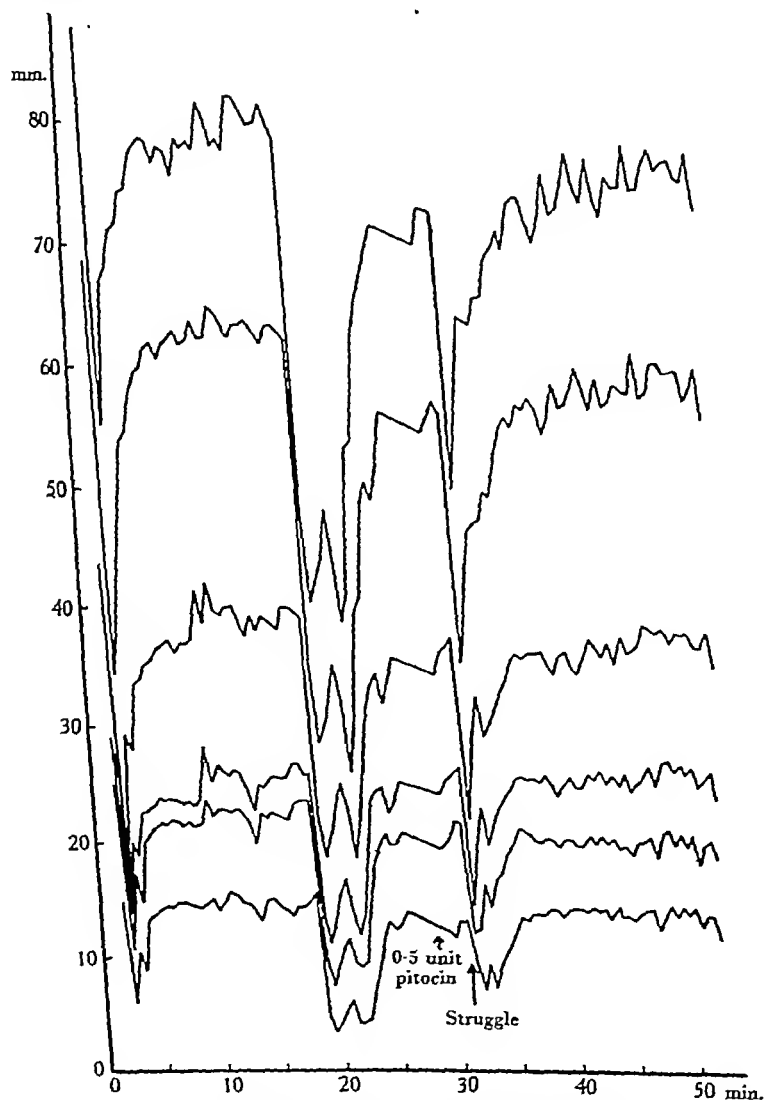


Fig. 4. Rabbit X 4. Stitches inserted 1 April 1937. Mare pituitary powder injected 17 July 1937. Experiment on 26 July 1937.

This dose would almost certainly have produced a marked effect had the usual methods of recording been used.

Sixteen days after the silver stitches had been inserted rabbit X 4 received intravenously 4.6 mg. of mare anterior pituitary powder (kindly given to me by Dr J. M. Robson) in suspension in saline. The X-ray photographs (Fig. 4) were taken 9 days later, which is about or just after the height of pseudopregnancy; this was confirmed by the very marked

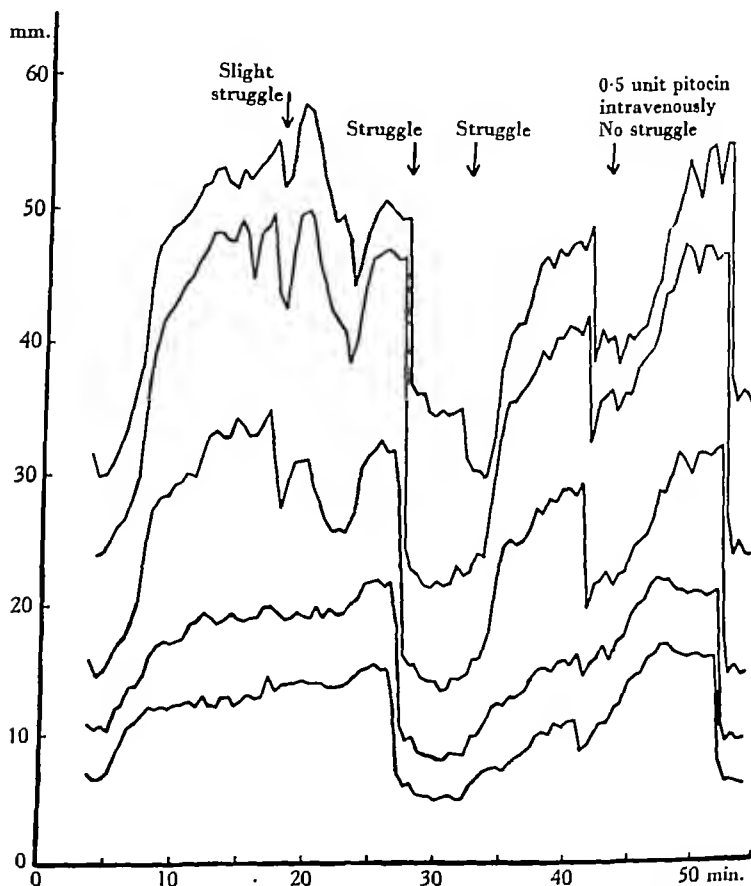


Fig. 5. Rabbit X 6. Spayed and injected with oestrone and progesterone.

progestational proliferation seen on histological examination of the uterus. The great length as compared with X 2 and X 3 is very obvious. The most striking point is the great extent of the spontaneous movements.

Numerous investigators, beginning with Reynolds & Friedman [1930], have reported that the rabbit uterus in pseudopregnancy is quiescent. In the case of X 4 the uterus was at one time less than half its maximum

relaxed length. The spontaneous contractions are separated by long quiescent periods of 10–15 min.; this is in contrast to the oestrin-treated rabbit, where contraction wave followed contraction wave without intermission. 0.5 unit of pitocin at 28 min. did not appear to have any effect; some time was lost in making this injection, this is indicated on the graph by the straight lines from 26 to 29 min.

As experiment X 4 showed such a great difference from the generally accepted state of affairs, it was decided to repeat the experiment in a slightly different way. Rabbits X 5 and X 6 were oöphorectomized and stitches were inserted in the left mesometrium. About 4 weeks later each was given a course of oestrone (0.01 mg. per day for a week) followed by 0.25 mg. progesterone (Proluton, kindly supplied by Schering Ltd.) twice daily for 4 days. The photographs were taken on the day after the last injection.

The two graphs are essentially similar (only that of X 6 is shown in Fig. 5). The uteri both showed marked progestational proliferation, but they were not quite so long as in the case of X 4, presumably because the latter had 9 days' progesterone treatment from its own ovaries as compared with 4 days' injection treatment in X 5 and X 6. As in the case of X 4, the graphs show very marked contractions separated by long quiet periods. In X 5 injection of 0.5 unit of pitocin was followed by a contraction of 4 min. duration which may have been spontaneous; in X 6 injection of 0.5 unit of pitocin was, unfortunately, given shortly after the beginning of a contraction which was smaller than the previous spontaneous ones. This absence of response to this relatively large dose of oxytocin is also seen when ordinary recording methods are used.

DISCUSSION

One of the apparent disadvantages of this method is that the detail of the curves is not so great as can be obtained by kymographic methods. If the curves are examined closely, it will be found that the omission of every other point would have altered very little the general trend; this simple test shows that photographs were taken frequently enough. Increasing the number of photographs in a given time would have added greatly to the cost of the experiment, and it would have increased the labour of measurement by requiring several hundred additional measurements for each graph without yielding any corresponding advantage.

The possible causes of the movements of the stitches are respiratory, intestinal and uterine. The outlines of the stitches are not quite sharp in every case, but in the vast majority the shadows are well defined in spite

of the exposure time being 2 sec. This rules out the respiratory cause as the rabbit breathes about 100 times per min.

The uterus and intestine were never adherent, but presumably the uterus might be pushed about by active coils of intestine. In the case of rabbit X 3 there was a very great difference between the trace obtained shortly-after spaying and that obtained a week later after oestrin treatment. The alteration in the character of the graph is almost certainly due to alterations in the behaviour of the uterus itself as the intestine is not affected by oestrin. Again, the speed of the movements, especially in X 4, X 5 and X 6, is very much slower than that of intestinal movements.

The shadows of two stitches may move towards one another by a contraction of the intervening part of the uterus or merely by an alteration in the angle formed by the line passing through the stitches and the horizontal, i.e. foreshortening. Consideration of the rabbit's uterus lying, as it does, on the abdominal wall and pressed down on it by the weight of the viscera would suggest that foreshortening, though possible, is not likely to be important. Further, in any big contraction it will be seen that, almost without exception, all the interstitch distances decrease simultaneously; it seems unlikely that the whole of the uterus would so regularly change its inclination to the horizontal simultaneously.

Since the rabbit has a very large and lax mesometrium, which permits very great mobility, the experiment was repeated on the cat which has a very short mesometrium. The uterus in this species is held close to the upper, i.e. dorsal, part of the abdominal cavity.

Cat X 2 was spayed and stitches were inserted along the left mesometrium. About 2 months later it was given 0.05 mg. of oestrone in oil twice a day for 10 days. At the end of this course of treatment it was decerebrated, and the anaesthetic allowed to blow off. The decerebration was performed because it was not thought likely that an unanaesthetized cat would lie quietly for an hour like the more docile rabbit. When marked decerebrate rigidity appeared photographs were taken. The graph (Fig. 6) shows two spontaneous contractions about 28 min. apart. Immediately after an injection of 0.5 unit of pitocin a large uterine contraction occurred in which the total length was reduced to nearly one-half of the maximum relaxed length. This contraction was more sustained than the spontaneous contractions. Immediately after the X-ray photographs were taken the uterine movements were recorded by means of the boat-shaped cannula described by Bell & Robson [1936]. The uterus now showed much greater spontaneous activity (Fig. 7); the X-ray method showed complete quiescence for about 17 min. between the two spontaneous contractions

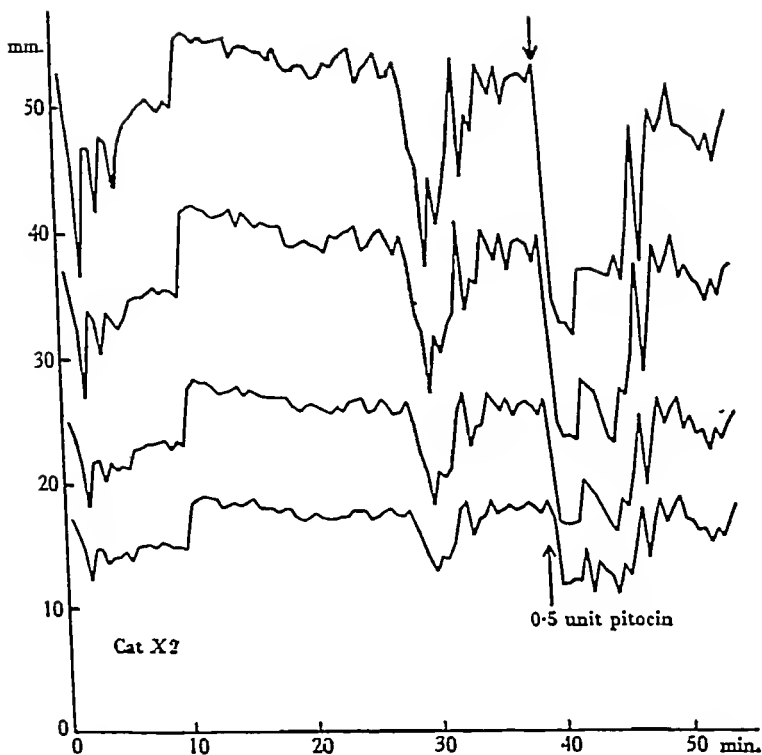


Fig. 6. Cat X 2. Injected with oestrone.

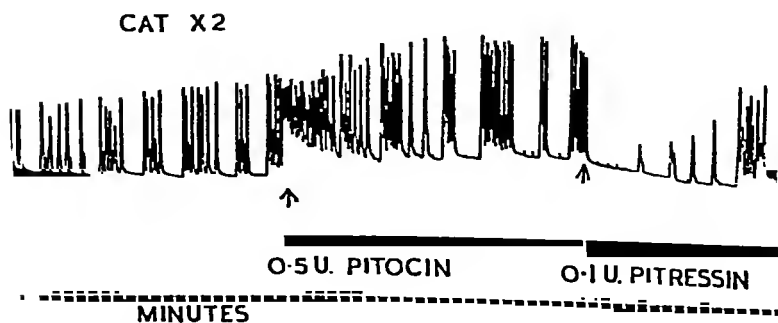


Fig. 7. Cat X 2. In this case the movements were recorded by a lever tied to the uterus and writing on a smoked drum. An upstroke denotes a uterine contraction.

while the maximum interval with the older method is 2 min.; the duration of the oxytocic action of pitocin is, however, of the same order. It is interesting to note that, while the reactions to oxytocin and vasopressin are as described by Robson & Schild [1938], the type of spontaneous activity is different. This may be due to the anaesthetic used by them or to the decerebration in the present case.

The finding that the uterine movements can be recorded by the present method even when the mesometrium is relatively tight, makes it even more certain that the movements found in the rabbit are genuine uterine movements on which the tightness or laxness of the mesometrium has little effect.

It would be most interesting to have records of the primate uterus in the unloaded state, because it is in this case that there has been most discussion of the effect of distension. Experiments on two *Macacus rhesus* monkeys were undertaken, but the graphs obtained were practically straight lines. The actual amount of contraction occurring in such a small fibrous uterus is very small, and may be masked by the movements of the uterus as a whole in the pelvis. The X-ray method gives practically no magnification and therefore cannot be expected to give even in large monkeys a good record of uterine movements.

CONCLUSIONS

There is no doubt now that the behaviour of the uterus *in vitro* is a most unreliable guide to its behaviour *in vivo*. The present work raises doubts as to whether all the work done on uterine movements *in vivo* can be accepted as it stands.

The most interesting finding in the present experiments is the large amount of spontaneous activity shown by the pseudopregnant rabbit uterus. In no other animal has complete quiescence been produced by oestrone and progesterone when the movements have been recorded by ordinary methods. If the present work is accepted, then an anomaly is removed.

It will be obvious that this X-ray method can be used only as a check on the more usual methods of determining uterine behaviour; it is very expensive and the making of measurements and graphs extremely laborious. If, therefore, the findings of this method agree with those of previous workers with other methods, then we must assume that the description already available is actually that of the behaviour in the normal animal. This appears to be the attitude to adopt to the experiments on rabbits X 2 and X 3. It seems that the rhythmic contractions

of the oestrous uterus are not excited by the weight of the recording lever, but the possibility that the spontaneous movements may be exaggerated by tension is not excluded, nor is any light thrown on the functional significance of these movements in the non-gravid uterus.

SUMMARY

A method of obtaining records of the movements of the uterus by taking serial X-ray photographs of silver wire stitches placed in the mesometrium along the uterine border is described. Graphs can be constructed to show the movements of the uterus in the absence of any tension or distension, and in the unanaesthetized animal.

The behaviour of the rabbit uterus as a result of spaying and after oestrin treatment is very like that described previously by ordinary kymographic methods. The uterus of a pseudopregnant rabbit shows long periods of quiescence with intervening large contractions. The uteri of rabbits treated with oestrone and progesterone show very similar movements. These uteri have been previously described as quiescent.

The behaviour of the uterus of a cat treated with oestrone is recorded. It shows rather less activity in the unloaded state as compared with the loaded.

The method is not applicable to the uterus of *Macacus rhesus*.

The possible causes of the variations shown on the graphs are discussed; it seems certain that they are true records of uterine movements.

I have to thank Prof. Cathcart for his interest in this work, Dr J. M. Robson and Dr J. S. Fulton for their advice on endocrinological and radiological matters respectively, Dr J. F. Harkness and Miss Patricia Clark who helped me with the graphical work. The expenses were defrayed out of grants from the Medical Research Council and the Rankin Fund of the University of Glasgow.

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AMINE OXIDASE IN *SEPIA OFFICINALIS*

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(Received 31 October 1940)

LITTLE is known of the amine metabolism in cephalopods. The experiments described in this paper are concerned with the occurrence in *Sepia* of amine oxidase, the system responsible for the inactivation of tyramine and other mono-amines. A few observations on the metabolism of diamines and on decarboxylases are also included.

The enzyme amine oxidase has chiefly been studied in vertebrates, but it also occurs in certain groups of invertebrates such as molluscs (*Mytilus*, *Patella*) and echinoderms [Blaschko, Richter & Schlossmann, 1937*b*; Blaschko, unpublished]. Its function in the mammalian body is not known, as no clear evidence for the occurrence of its substrates such as tyramine and isoamylamine, exists. When adrenaline was found to be a substrate of the enzyme *in vitro* [Blaschko *et al.* 1937*a*], it was suggested that it might be the chief substrate of the enzyme *in vivo* [see also Gaddum & Kwiatkowski, 1938], but it seems doubtful whether this view can be maintained. In the invertebrate it is obvious that the enzyme is not associated with the metabolism of adrenaline, since the oxidase does not occur in annelids (e.g. *Lumbricus*), which contain chromaffine tissue, and adrenaline has never been detected in molluscs and echinoderms.

The present study was undertaken because tyramine is said to occur in cephalopods, and to play a role as a hormone in their regulative processes. Tyramine was isolated from the posterior salivary glands in *Octopus* [Henze, 1913], and appears to be one of the poisonous substances present in the saliva of cephalopods [Bottazzi & Valentini, 1924]. According to Sereni [1930], these glands may be considered as endocrine organs which not only excrete tyramine but also release it into the circulation.

METHODS AND MATERIAL

The experiments were carried out on tissue extracts of *Sepia officinalis*, which is caught in great numbers at Arcachon. The animals were dissected shortly after being caught. The organs were ground with sand and $M/15$ sodium phosphate buffer solution (pH 7.4) was added. Usually, equal amounts of organ and of phosphate solutions were taken, but in a few cases less tissue was used. The suspensions were centrifuged for 5 min. and the supernatant fluid was used for the experiment. The chief digestive gland, the liver, is easily dissected. The so-called "kidneys" represent a loose glandular structure, mixed up with blood vessels; no attempt at separation of these tissues was made. The muscle was taken from the mantle. The wall of the ink sac was used after removing the contents and repeated washing; but even then the tissue did not lose its black colour, and the extracts looked intensely dark. The ink gland forms part of the wall of the sac; it was used together with the rest of the wall.

The amine oxidase activity was determined in the presence of semicarbazide, in order to fix the aldehyde formed in the reaction, and to prevent uptake of oxygen from its further oxidation. The activity was measured by determining the extra amount of oxygen used after the addition of substrate. The activity is expressed in c.mm. O_2 /g. fresh tissue/hr. Usually tyramine was the substrate; sometimes isoamylamine and $(-)-p$ -sympatol ($C_6H_4OH.CHOH.CH_2NHCH_3$) were also tested. The oxygen uptake of the extracts was determined manometrically, as previously described [Bhagvat, Blaschko & Richter, 1939]. The flasks—conical flasks of the Barcroft-Warburg type—were prepared as follows:

Main flask: 1.6 c.c. enzyme preparation + 0.2 c.c. $M/2$ semicarbazide hydrochloride.

Side bulb: 0.2 c.c. $M/4$ amine hydrochloride.

Inner tube: 0.3 c.c. N KOH.

In a few experiments in which extracts were tested for the presence of histaminase (or diamine oxidase) histamine and putrescine were used as substrates; but no semicarbazide was added since it inhibits the enzyme responsible for the oxidation of these substrates.

RESULTS

In Table I the oxygen uptake of various tissues is given with tyramine as substrate. Apart from muscle, all the tissues examined showed a considerable oxygen uptake. The highest activity was observed with extracts of liver, which exceeded even that of the mammalian liver. In

TABLE I. Amine oxidase activity of various organs of *Sepia* with *M*/40 tyramine, measured as o.mm. O₂ taken up/g. fresh tissue/hr.

Animal	Organ	Oxygen uptake	Temperature
<i>Sepia</i>	Liver	657	19.0
"	"Kidneys"	246	21.0
"	Posterior salivary gland	246	19.5
"	Ink sac	173	18.5
"	Muscle	0	19.5
Guinea-pig	Liver	486	37.0

the last row of the table the corresponding figure for the amine oxidase activity of the guinea-pig's liver, as determined at 37°, is given for comparison.

The conclusion that the oxygen uptake in these experiments really resulted from the action of amine oxidase is supported by the fact that the oxidation of tyramine by extracts of *Sepia* liver was abolished in the presence of *sec.*-octyl alcohol, known to be a strong inhibitor of amine oxidase. On the other hand, neither cyanide nor semicarbazide inhibited the oxidation, which again is in agreement with the observations on the mammalian enzyme.

*iso*Amylamine and (-)-*p*-sympatol were used as substrates for the extracts from liver, and were found to be oxidized. These experiments revealed a characteristic difference in the activities of preparations from *Sepia* and from mammals. Whereas the mammalian enzyme showed no marked preference for tyramine as compared with *iso*amylamine, in *Sepia* the rates were greatly in favour of tyramine. A similar observation has been reported for the amine oxidase of the gastropod *Patella* [Blaschko *et al.* 1937*b*]. In Table II the relative rates of oxidation of

TABLE II. Comparison of the relative rates of oxidation of tyramine, *iso*amylamine, and (-)-*p*-sympatol

Animal	Organ	Relative rate of oxidation of		
		Tyramine	<i>iso</i> Amylamine	(-)- <i>p</i> -Sympatol
Guinea-pig	Liver	100	105	48
<i>Sepia</i>	Liver	100	24	12
<i>Patella</i>	Viscera	100	20	13

*iso*amylamine, (-)-*p*-sympatol, and tyramine are given, that of the latter being taken arbitrarily as 100. In the table are included figures for *Patella*, similarly calculated, taken from the paper of Blaschko *et al.* The relative oxidation rates for the three substrates in the extracts from the two molluscs agree with each other, but they differ from those found in the guinea-pig. The enzymes from both *Sepia* and *Patella* oxidized

tyramine much more rapidly than the other two amines, whereas the preparation from the mammalian liver oxidized isoamylamine and tyramine at about the same rate. (-)-*p*-Sympatol was less rapidly oxidized in all extracts, but again the figures for *Patella* and *Sepia* agree with each other, and differ from those for the guinea-pig.

Oxidation of diamines. No evidence for the presence of histaminase (diamine oxidase) in *Sepia* has been obtained. The experiments were carried out with preparations of liver, histamine and putrescine being used as substrates, and with extracts of the "kidneys" and putrescine as substrate. There was no extra uptake of oxygen on the addition of the amines.

Experiments on decarboxylases. The claim that tyramine occurs in cephalopods led us to inquire whether there exists in *Sepia* an enzymatic mechanism for decarboxylating *l*(-)-tyrosine. As the enzyme which decarboxylates the closely related amino acid *l*(-)-dopa [Holtz, Heise & Lüdtke, 1938] is very specific for this substrate, and does not decarboxylate *l*(-)-tyrosine [Blaschko, 1939], we have also searched for the presence of this enzyme in *Sepia*. Extracts from liver, "kidneys", and the posterior salivary glands were used. They were found incapable of decarboxylation, no CO₂ being formed when *l*(-)-tyrosine or *l*(-)-dopa were added.

DISCUSSION

Extracts of the tissues of *Sepia* show an uptake of oxygen when incubated with tyramine and other amines. There can be little doubt that the oxygen uptake observed is due to amine oxidase. All the characteristic properties of the enzyme, such as the inhibition by octyl alcohol, the lack of sensitivity to both cyanide and semicarbazide are present in the preparations from *Sepia* as well as in those from the guinea-pig. The difference in the relative rates of oxidation of different substrates may be explained as follows. The affinity of an enzyme to its substrate is determined not by the prosthetic group of the enzyme molecule, but by the specific protein to which this group is attached. In the case of amine oxidase, the two preparations from the more closely related animals show the same relative oxidation rates, but they differ from the vertebrate preparation. The protein constituents of the enzymes from the two molluscs are probably more closely related to each other than they are to the protein in the vertebrate enzyme. That such differences in affinity are due to the proteins, is in agreement with conceptions of substrate specificity [see Warburg & Christian, 1938].

Sepia is known to contain a tyrosinase [Przibram, 1902], but the oxidation of tyramine cannot be attributed to this enzyme, as the tyrosinase from *Sepia* oxidizes tyrosine, but not tyramine [Neuberg, 1908]. This was confirmed in manometric experiments. Moreover, the action of inhibitors on the oxidation—sensitivity to octyl alcohol, insensitivity to cyanide and semicarbazide—seems to rule out the possibility that a phenolase contributed to the oxidation of tyramine.

It seems doubtful whether Henze's [1913] observations can really be accepted as satisfactory evidence for the occurrence of tyramine in cephalopods. However, in connexion with his findings and Sereni's [1930] experiments, it is interesting that *Sepia* contains a powerful mechanism for the inactivation of tyramine, and the marked preference for tyramine makes it likely that this amine is the main substrate for the enzyme in the living animal. But the evidence available must be considered as insufficient. Furthermore, our experiments with decarboxylases have given no evidence for the presence of a tyrosine decarboxylase. It would be desirable to repeat these experiments on octopods, the animals used by Henze, but they were not at my disposal.

The inability of the extracts from *Sepia* to oxidize histamine and putrescine, in spite of their strong amine oxidase activity, demonstrates again the different role of the mono-amines and di-amines in metabolism. It may be mentioned that histaminase has up to the present not been found in any invertebrate tissue.

SUMMARY

1. Extracts from various tissues of *Sepia officinalis* show that amine oxidase is present in the liver, the posterior salivary glands, the "kidneys", and the wall of the ink sac, but not in muscle. The highest activity was found in the liver, the activity being greater than in mammalian liver.

2. Unlike the mammalian enzyme, the amine oxidase of *Sepia* shows a marked preference for tyramine over other substrates. This may perhaps be taken as evidence in favour of the belief that tyramine plays some role as a hormone in cephalopods.

3. No evidence for the presence in *Sepia* of histaminase, or of a decarboxylase for either *l*(-)-tyrosine or *l*(-)-dopa was obtained.

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THE EFFECT OF THE LEVEL OF INORGANIC BASES
IN BLOOD ON THE CATABOLISM OF FOOD PROTEIN

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IN the course of an investigation [Harris, Ireland & James, 1937] which had for its object the relation of protein intake to high blood pressure it was established that an excess of protein in the diet raises systolic pressure. A recent paper by Verney & Vogt [1938] supports this. It was also established [Harris *et al.* 1937] that a large intake of protein leads to retention of nitrogen in the human body. In cases where there is pronounced retention of nitrogen it was found that the level of blood plasma nitrogen, as well as whole blood total nitrogen, is higher than when the same subject takes a low protein diet. This fact is of some significance, as in cases of high blood pressure the range of protein plasma values is frequently higher than in normal subjects. High protein values in whole blood and plasma may increase the viscosity of the blood and must raise the colloidal osmotic pressure in the circulation; this may be a possible factor in the aetiology of hypertony.

The present investigation has for its object a comparison of the effect of high and low intake of bases on the total nitrogen of the blood whilst protein intake is constant. It must be borne in mind that a very low nitrogen intake might not be sufficient to meet the wear and tear of the tissues. In such circumstances the nitrogen output will be greater than the intake. On the other hand, with a large nitrogen intake there is retention of nitrogen. Other things being equal, the larger the nitrogen intake the lower the nitrogen output in relation to the intake. In addition the relationship of the intake of inorganic bases to the bases of the blood and the excretion of nitrogen and bases in the urine has been followed.

METHODS

The subjects of our investigations received the same amount of protein for two periods: in one with high inorganic base, and in the other low. Determinations were made of (a) the nitrogen balance between

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intake and output; (b) the level of total blood nitrogen during the two periods; (c) the balance of bases between intake and output; (d) the blood level of the bases in the two periods.

Subjects used and dietary periods

A group of patients was fed during alternate periods on high and low bases respectively, whilst remaining during the two periods on the same protein intake. Each period lasted about a fortnight. Between the two periods 3 days of a standard low protein diet were interposed. The urine was examined daily: each period therefore represented fourteen determinations. Blood was examined twice weekly. The data obtained from the first 2 days of the period in which the patient was put on a particular diet were not included in the averages. High and low base diets were given whilst the patients were receiving (a) a moderate protein intake (about 10 g. nitrogen per day); (b) a high protein intake; (c) a protein diet of about 5 g. nitrogen. In some cases the period of low base was taken first, in others the period of high base. The calorie values and other conditions, such as fluid intake, were about the same during the two periods.

The patients who were subject to these investigations were suffering from high blood pressure or valvular disease, but they exhibited no evidence of heart failure. From the point of view of this investigation they must be looked upon as normal cases. It was not aimed in this investigation to obtain absolute nitrogen minima: all that was attempted was to compare under similar conditions the effect of high and low base diets on protein metabolism.

Diets

The following are particulars regarding the daily diets:

High base diet. 600 c.c. milk; 80 g. salt butter; 120 g. salt bread; 100 g. ham; 50 g. bacon; 100 g. tinned pears; 50 g. sugar; 50 g. jam; 1 egg; 100 g. stewed prunes; 100 g. chipped potatoes; 100 g. carrots; 100 g. beetroot.

Low base diet. 80 g. single cream; 60 g. clotted cream; 80 g. salt-free butter; 120 g. salt-free bread; 150 g. beef; 100 g. tinned pears; 100 g. sugar; 50 g. jam; 1 egg; 100 g. stewed prunes; 100 g. mashed potatoes; 100 g. cauliflower; 30 g. cream crackers.

Added bases. Ca as 13.5 g. Ca saccharate and K as 7 g. KCl (= 1.35 g. Ca and 3.66 g. K).

In some instances we relied solely on diet containing high and low bases; in others bases were added to the food.

Methods of sampling

Foods. The food analyses used were the average of a number of analyses carried out at different times on the actual foods as supplied to the subjects.

Bloods were taken fasting (i.e. before breakfast) twice weekly from the median basilic vein.

Urines—analysed in full 24 hr. specimens, all urine passed by subjects being collected and preserved with toluene.

"Percentage N output" refers to urine total N expressed as a percentage of (food—faecal) N.

Analytical methods

Total N	Kjeldahl method with the Arnold-Gunning digestion method.
Sodium	Iodometrically as pyroantimonate.
Potassium	As cobaltinitrite.
Calcium	Titrimetric as oxalate.
Carbohydrates in foods	By the use of the polarimeter.
Fats in foods	Soxhlet and Rose-Gottlieb methods.
Non-protein nitrogen (blood)	Folin and Wu's method.
Acidity of urine	Folin's method.
Nitrogen partition in urine:	
Urea and ammonia	Van Slyke and Cullen's method.
Amino-acids	Formaldehyde titration.
Creatinine	Folin's colorimetric method.
Uric acid	Benedict's colorimetric method.

RESULTS

High and low base on 10 g. nitrogen

Nitrogen output in relation to intake. The average daily nitrogen output in the urine reckoned as a percentage of the intake was determined in fourteen subjects whilst receiving the high and low base diet (Table I). The ratios of the N output on high base to that on low base calculated as a percentage varied from 129.4 to 94.2, the average being 108.5 ± 2.8 (s.e. of mean of fourteen cases). This indicates that the N excretion is significantly higher on the high base diet.

TABLE I. This gives the average percentage output of nitrogen in relation to intake on high and low base diet

Patient	Average nett N intake g./day		Average N output (as a percentage of nett intake)	
	High base	Low base	High base	Low base
As.	9.27	9.21	105.1	81.2
M.	9.08	8.92	84.0	79.1
Th.	9.098	9.04	88.5	75.3
Mg.	8.68	9.64	107.5	90.2
F.	9.07	9.64	98.0	91.8
Mv.	8.47	8.83	106.3	98.4
Tn.	9.07	8.87	103.5	107.2
S.	8.33	8.12	154.6	153.0
Mu.	8.61	8.10	99.1	105.2
An.	8.03	8.52	87.7	86.3
Me.	8.71	7.66	61.7	61.2
P.	8.10	8.66	100.3	78.3
C.	9.26	9.41	91.4	82.6
W.	7.99	8.92	75.3	68.6

In this group of cases the protein intake contained about 10 g. of nitrogen daily during the whole time of the experiments; although it will be seen that in some cases the nitrogen intake on low and high base diet is not absolutely the same. The calories varied but little during the two periods.

TABLE II. This shows the difference of total N in blood on low and high base diet

Patient	Average blood total N g./100 c.c.		Average blood non-protein N g./100 c.c.	
	High base	Low base	High base	Low base
As.	2.997	3.10	29.1	28.7
M.	2.15	2.21	32.5	28.1
Th.	2.99	2.903	28.7	25.6
Mg.	3.185	3.36	29.5	27.0
F.	3.30	3.456	29.3	35.1
Mv.	3.364	3.486	27.0	27.5
Tn.	3.518	3.471	31.3	26.9
S.	3.044	3.005	28.9	30.5
Mu.	3.094	3.299	29.9	28.4
An.	3.168	3.216	29.3	29.4
Me.	3.221	3.354	26.7	23.8
P.	3.400	3.357	25.9	27.8
C.	3.275	3.254	25.7	26.4
W.	2.574	2.763	57.8	70.0

Total nitrogen and non-protein-nitrogen of blood. Table II gives the average total nitrogen figures of whole blood for the fourteen cases referred to in Table I. (The N.P.N. is also given.) The ratios of total nitrogen on low base diet to that on high base diet calculated as a percentage give:

Max. 107.4 %
Min. 97.1 %

With av. $102.7\% \pm 0.89$ (s.e. of the mean of the fourteen cases).

It will therefore be seen that the total N of the whole blood is higher on low than on high base diet.

It is clear that the N of the whole blood stands in inverse relation to urinary nitrogen. It is interesting to see that the N.P.N. does not run parallel with the total N figures. There is of course no necessity for such a parallelism.

Percentage output of base in relation to intake. Table III gives the average intake and output and percentage output of inorganic base for the fourteen cases on high and low base diet. The total output of base is of course lower on low than on high base diet. On the other hand, on a low base diet the percentage of output to intake is definitely higher in regard to potassium and calcium but not in regard to sodium. The ratios of percentage output on low base diet in relation to that on high base diet give:

Calcium:	Max.	435.5 %
	Min.	91.2 %

With av. $236.2 \% \pm 25.3$ (s.e. of mean of fourteen cases).

Potassium:	Max.	410.2 %
	Min.	186.7 %

With av. $271.1 \% \pm 29.4$ (s.e. of mean of fourteen cases).

Sodium:	Max.	206.0 %
	Min.	37.0 %

With av. $125.5 \% \pm 12.8$ (s.e. of mean of fourteen cases).

Bases in blood. Table IV gives the average amounts of inorganic base in the blood in the fourteen cases. Our figures of calcium are too few to be of value and those of sodium show no significant variation. The figures for ratios of blood potassium on high base diet in relation to that on low base diet calculated as a percentage work out as follows:

Blood potassium:	Max.	126.3 %
	Min.	100.8 %

With av. $108.5 \% \pm 2.07$ (s.e. of mean of twelve cases).

The potassium values are higher therefore on high base diet.

This fact is of considerable interest. Elsewhere [Harris *et al.* 1937] we found that where the potassium intake is about the same a higher nitrogen in the blood accompanies a higher potassium value. A high total blood nitrogen thus runs parallel with a high blood potassium. In

TABLE III. This shows output of bases in comparison to intake on 10 g. N

Patient	Average intake		Average output		Average output (urine and faeces)	
	g.		g.		%	
	High base	Low base	High base	Low base	High base	Low base
Sodium						
As.	2.428	1.036	2.575	1.146	106	110.6
M.*	2.304	1.034	3.083	1.112	133.8	107.5
Th.*	2.336	1.024	2.199	0.925	94.2	68.1
Mg.	2.76	1.04	3.131	0.468	113.5	42.0
F.	2.76	1.04	2.945	1.364	106.7	131.1
Mv.*	2.66	1.039	2.722	1.035	102.2	99.7
Tn.	2.74	1.036	1.900	1.117	69.3	107.8
S.	2.71	0.938	1.085	0.953	73.3	96.4
Mu.*	2.263	0.888	1.864	0.919	82.3	103.5
An.*	2.55	1.035	2.215	1.683	78.0	160.7
Me.	2.577	0.893	1.749	1.227	67.9	137.5
P.	2.75	1.01	2.210	1.147	80.4	113.6
C.*	2.413	1.04	1.463	1.160	60.8	102.5
W.	2.574	1.04	2.135	0.971	82.9	93.3
Potassium						
As.	6.23	2.222	3.162	2.092	50.4	94.1
M.*	9.77	2.203	3.895	2.491	39.8	110.6
Th.*	9.775	2.143	3.425	2.259	35.8	105.1
Mg.	6.3	2.24	3.253	2.326	51.6	103.8
F.	6.3	2.24	2.806	2.200	44.5	98.2
Mv.*	9.53	2.23	2.847	2.489	29.8	111.5
Tn.	6.3	2.24	2.428	2.063	38.5	92.0
S.	6.125	1.953	2.394	1.922	41.0	98.3
Mu.*	8.394	1.99	2.222	2.158	26.4	108.5
An.*	9.777	2.222	3.237	1.931	33.2	86.8
Me.	6.24	2.067	2.160	1.976	37.0	95.6
P.	6.243	2.197	2.758	2.377	45.2	108.2
C.*	9.952	2.24	2.676	1.849	27.0	83.3
W.	6.296	2.24	2.403	2.120	31.5	91.1
Calcium						
As.	1.176	0.400	1.226	0.605	104.2	151.2
M.*	2.31	0.397	1.577	0.507	68.2	127.6
Th.*	2.323	0.380	1.401	0.526	60.9	138.4
Mg.	1.22	0.400	0.882	0.819	72.2	204.7
F.	1.22	0.400	0.821	0.468	67.3	117.0
Mv.*	2.48	0.404	1.063	0.580	42.8	143.5
Tn.	1.219	0.400	0.678	0.335	55.6	84.3
S.	1.205	0.378	0.953	0.521	79.0	137.9
Mu.*	2.192	0.365	0.990	0.447	45.2	122.6
An.*	2.474	0.393	0.500	0.560	32.3	140.7
Me.	1.205	0.382	0.899	0.637	67.1	166.7
P.	1.217	0.590	0.653	0.478	55.3	122.5
C.	2.46	0.400	0.818	0.479	33.2	119.8
W.	1.202	0.400	1.618	0.490	134.5	122.6

Patients marked thus (*) received added bases in the form of potassium chloride and calcium saccharate.

Table IV, although the whole blood N is lower on the high base diet the potassium values are higher. These two findings are not contradictory but simply imply that retention of potassium on high base diet is a more pronounced factor than a low blood N.

TABLE IV. This shows blood Na and K on high and low base diets

Patient	Average blood bases			
	Sodium mg./100 c.c.		Potassium mg./100 c.c.	
	High base	Low base	High base	Low base
As.	301.2	302.5	22.6	21.5
M.	321.6	304.9	21.5	20.9
Th.	302.9	317.5	24.3	22.9
Mg.	302	306.5	21.9	21.7
F.	315	307.5	21.1	—
Mv.	304	300.0	24.0	21.8
Tn.	315	304	23.8	22.5
S.	308	314	23.4	20.8
Mu.	316	307	23.8	22.0
An.	307	298.3	23.6	20.5
Me.	304	305.1	24.0	23.8
P.	311.8	318.3	24.5	19.4
C.	307	318.5	—	22.9
W.	309.6	318.5	24.6	20.8

High and low base under low protein

Experiments on high base intake and high protein were unsatisfactory. We had difficulty in getting all the patients to take a standard high protein diet. Some patients took a great deal more than others. Owing to the fact that the intake of protein was not the same during the two periods the results are not comparable and are therefore not given. Our experiments with high and low base intake on low protein diet are also

TABLE V. This gives the percentage output of bases in relation to intake when the protein intake is low

Patient	Average intake		Average output		Average output (urine and faeces)	
	g.		g.		%	
	High base	Low base	High base	Low base	High base	Low base
	Sodium					
Hs.	1.60	0.43	0.882	0.656	55.1	152.4
Ho.	1.76	0.43	1.383	0.911	78.6	211.8
Mc.	1.62	0.42	1.034	0.588	63.8	140.0
S.	1.75	0.43	1.160	0.662	60.6	153.0
	Potassium					
Hs.	6.81	2.07	1.527	1.644	22.4	79.4
Ho.	7.07	2.08	2.010	1.964	28.4	94.8
Mc.	7.14	2.04	1.573	1.425	22.1	69.9
S.	7.08	2.08	2.149	2.210	30.4	106.3
	Calcium					
Hs.	1.87	0.25	0.477	0.364	25.4	145.6
Ho.	2.04	0.24	0.741	0.487	36.7	202.9
Mc.	2.00	0.25	0.513	0.405	25.6	162.6
S.	2.03	0.25	0.656	0.404	32.3	161.6

omitted, because we are dealing here with relatively small quantities of protein intake, and the difference in the amount of nitrogen retained on the two diets can only be slight. However, there is still at least as pronounced a retention of base on high base low protein diet as on a 10 g. of nitrogen intake (Table V)—a matter of some importance as will be seen from the discussion.

DISCUSSION

The evidence that inorganic bases influence the level of nitrogen in the whole blood is indicated by the following: Under high base intake there is (a) a lowering of the total nitrogen of the blood; (b) an increased elimination of nitrogen; (c) a rise of potassium in the blood, and (d) a lower percentage elimination of bases. Although the number of cases is small, each set of figures shows a significant statistical difference and they thus supplement and support each other.

No significant difference in the figures was obtained for blood sodium on low and high base diet. In this and other investigations we usually found that its value in the blood is less dependent on the intake of the substance than in the case of other bases. An excessive sodium intake rapidly finds its way to the tissues even before it can be eliminated by the kidney, and the figures in Table IV for blood sodium do not show any constant or consistent values. In another investigation, too, we found it difficult to raise the blood sodium by the administration of large doses of sodium chloride.

Berg [1931] showed that bases influence protein metabolism. It has been established in this investigation that the large intake of base lowers the total nitrogen values of the whole blood, in consequence of which a larger proportion of protein intake is catabolized to the usual end products of protein metabolism.

Whatever the factors which are responsible for the replacement of part of nitrogen in the blood by base on high base intake, it appears that the pronounced retention of base on high base intake is not entirely dependent on the level of nitrogen in whole blood; for as we have seen on a low protein intake when the displacement of nitrogen is slight or does not take place at all, retention of base is quite pronounced. We may assume that on low base diet the bases are sufficient for the requirements of the organism, since the output almost balances the intake. Possibly the retention of base takes place on a high base intake in the same way as we have found the retention of nitrogen on an excessive protein intake. We surmise that in the latter, not only is the nitrogen raised in the blood

as a result of excessive intake, but also in the tissue cells, because the amount of nitrogen retained is larger than could be accounted for by increase of nitrogen in the blood alone. We may be dealing here with a law governing retention and storage of food, base when offered in large quantities being retained in preference to protein. Whatever the explanation may be, the facts established in this paper are of interest from several points of view. The level of protein and base in the blood may influence the colloidal osmotic pressure, pH, viscosity, blood volume, heart contraction and exchange of fluid between tissue and circulation.

There is a considerable base retention on a high base intake. We know that Ca is easily stored, but even the K retention is not greater than the normal fluctuation values of K in the tissue.

SUMMARY AND CONCLUSIONS

1. The percentage nitrogen output in the urine on a moderate protein intake (10 g. N) is lower on low base diet than on high.
2. The total N of the whole blood is higher on low base than on high base diet, the nitrogen of the whole blood standing in inverse ratio to urinary nitrogen output.
3. Potassium in blood is higher on high base diet than on low.
4. The percentage of output in relation to intake of base is higher on low base than on high base diet.

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NEURO-MUSCULAR TRANSMISSION IN THE EXTRINSIC MUSCLES OF THE EYE

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THE extrinsic ocular muscles are peculiar among the voluntary muscles of the mammal in their sensitiveness, while normally innervated, to substances with nicotine-like effects, even when these reach them slowly through the blood stream, or are applied to the surface of the surviving muscle suspended in Locke's solution [cf. Duke-Elder & Duke-Elder, 1930]. In this respect, they are similar to the skeletal muscles of birds. The muscle of the fowl, however, shows, in addition to its responses to acetylcholine and eserine, certain other interesting differences from mammalian muscle in neuro-muscular transmission [Brown & Harvey, 1938*a, b*]. It appeared probable, therefore, that the mammalian eye muscles might exhibit properties intermediate between mammalian and avian skeletal muscles, and provide opportunities of elucidating some of the more obscure features of the effects of the substances affecting neuro-muscular transmission.

METHODS

In all the experiments we used cats, decerebrated under ether anaesthesia, and at least 1 hr. was allowed to elapse between the removal of the anaesthetic after decerebration and the beginning of the record from an eye muscle. In a few of the early experiments, we stimulated the III nerve within the cranial cavity and recorded from the internal rectus muscle, but we abandoned this technique in favour of exposure of the III nerve in the orbit, since it is difficult to free for stimulation a satisfactory length of the intracranial portion of the nerve, and what is available is extremely fragile. The necessary dissection towards the sphenoidal fissure may, moreover, compromise the blood supply of the

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orbital structures. The most satisfactory preparation proved to be the inferior oblique muscle, the nerve to which has a long intraorbital course.

As soon as the decerebration was finished, and before the occluding clamps had been removed from the vertebral and carotid arteries, we cut the ophthalmic division of the V nerve at its entry to the Gasserian ganglion on the side from which records were to be taken, usually the left. This procedure was of assistance in the preparation of the muscle and nerve. If it was omitted, reflex head movements made difficult the dissection of the delicate orbital tissues, and the reflex rise of blood pressure, which resulted particularly from removal of the globe of the eye, was apt to start a severe haemorrhage from the carotid and vertebral stumps within the skull. The lower eyelid was then removed, and the bone of the lower margin of the orbit was excised to the extent of about 1.5 cm. lateral and 1.0 cm. ventral to the inner canthus. The conjunctival sac was incised and the tendon of the inferior oblique muscle was tied with a fine silk thread and lifted away from the globe of the eye. This enabled the nerve to be identified and freed from connective tissue. The globe was then eviscerated, retracted, and tied as far back as possible. The dissection of the nerve into the depths of the orbit was continued until the ciliary ganglion could be seen. This was seized with fine forceps and its connexions, other than the nerve to the inferior oblique muscle, were cut. This procedure provides a nerve of some 1.5 cm. in length, and both nerve and muscle retain their excitability for several hours. The nerve was laid on a pair of fine platinum wire electrodes 2 mm. apart carried in a manipulator, the stimulating cathode being approximately 1 cm. from the muscle.

For myographic recording, the animal's head was immobilized by screwing the threaded end of a steel bar into a hole drilled and tapped in the parietal bone, and fixing the bar to a clamp on the myograph stand. The silk thread, tied to the tendon of the inferior oblique muscle, was fastened to a very weak torsion-wire myograph, mounted on the same stand. The electrical records were taken from fine silver pins, one, an earthed lead inserted in the muscle at, or about the point of entry of the nerve, and the other, the grid lead of the input, at the musculo-tendinous junction. We found that monophasic recording was only obtainable by killing the extreme end of the muscle by the application for a few seconds of a small ball of fused KCl. The killing of the end usually had to be repeated in the course of an experiment. The amplifier was the one used in previous experiments [cf. Brown & Euler, 1938], the output supplying either a Matthews oscillograph or a cathode-ray oscillograph.

The stimuli were taken from the secondaries of air-cored transformers actuated by condenser discharges from gas discharge tube circuits [Schmitt & Schmitt, 1932]. They were timed by a Lucas pendulum.

In all experiments the temperature and humidity of the preparation was maintained by enclosing the animal's head in a celluloid box, with a skirt of sheet rubber. The air in the box was warmed and kept moist by a 36 W. lamp covered by a wick dipping into water, the temperature being kept at approximately 38° C.

In a few experiments, we took records from the superior oblique muscle, stimulating the IV nerve between its cranial origin and its disappearance into the dura mater covering the edge of the tentorium cerebelli.

In the study of the effects of denervation, cats were anaesthetized with "Dial", and, with full asepsis, the skull was trephined, and the dura mater opened. The left cerebral hemisphere was gently lifted to expose the III nerve between the base of the mid-brain and its entry into the dura. It was divided by pulling it free from its origin in the mid-brain with a blunt hook. The animals made an uneventful recovery.

Drugs were administered either intravenously or by injection into the carotid artery, through a cannula tied in the central stump of the severed lingual artery. At the moment of injection the carotid artery was occluded by a clamp placed central to the origin of the lingual artery. The clamp was removed as soon as the injection was completed.

Acetylcholine chloride and eserine sulphate were freshly prepared in saline acidified to pH 4. Curarine chloride [King, 1935] was used for producing neuromuscular block.

RESULTS

Normal responses to nerve stimulation

The tension response of the muscle to single nerve volleys is so rapid in all its phases that accurate recording of the changes in tension with the ordinary mechanical myograph is impracticable. Our measurements indicate that the peak of the twitch-tension may be attained in as little as 7-8 msec. when the muscle is at a temperature of 39° C., and half relaxation is reached in 6.5-7 msec. from the peak. The highest tension maximum attained by a cat's inferior oblique muscle was 1.6 g. The average of five experiments was 0.96 g.

The electrical response to a single nerve volley is, similarly, of brief duration and is nearly symmetrical in rise and fall, the crest being

attained in 0.9–1.0 msec., and the return to equipotential level taking from 1.2 to 1.4 msec. (cf. Fig. 7*a, b*). Exact determination of the time of the return is difficult on account of the slow potential which follows the spike. In most of our experiments on the inferior oblique muscle, the action potential has appeared homogeneous, there being few signs of any fibres having characteristics outstandingly different from the majority. In one experiment on the superior oblique muscle, we observed a much slower wave, succeeding the main action potential, which could be brought into prominence by altering the strength of the stimulus applied to the nerve. It appeared, strangely enough, to be associated with a nerve fibre group having a lower threshold than those supplying the faster fibres of the muscle. Apart from occasional divergences such as this, the action potentials of the eye muscles indicate that the fibres respond with very little temporal dispersion, for the duration of the whole potential is very little longer than the duration of a minimal response to a nerve volley, e.g. in one experiment the duration of a maximal response was 2.8 msec., and of a minimal response, forty times less in amplitude, 2.4 msec. In addition to the spike potential, we have frequently observed a slow negative potential succeeding it. If the responses are truly monophasic, the slow potential appears as a prolongation of the descending limb of the spike. The magnitude of the slow negativity has varied considerably and, apart from the necessity of monophasic recording, we have not yet found the conditions which lead to its prominence. In one experiment, on a cat anaesthetized with ether, the slow potential had a voltage of nearly half that of the spike voltage. We can give no accurate figure of the duration of the slow potential, as these records have been taken with a condenser-coupled amplifier, but a rough estimate suggests that they persist for 10–20 msec. after the spike is over.

Response to two nerve volleys

The tension response to two nerve volleys, about 5 msec. apart, is some 2.5 times that of the response to a single volley. This compares reasonably well with the figure of "more than 3" given by Cooper & Eccles [1930] for the single-response/double response ratio in the internal rectus of the cat. Muscular summation begins when the nerve volleys are separated by 0.4–0.6 msec. and, when once the second response has occurred, the curve relating tension to interval between stimuli rises steeply. At a stimulus interval of 2.5 msec. summation is almost maximal, only a slight further increase occurring as the interval is lengthened. With stimuli further apart than 5 msec., the two responses become dis-

crete, on account of the very short duration of the individual twitch. Accurate determinations of the refractory period from the muscular summation curve are difficult, on account of the deficiencies of mechanical recording, and most of our measurements have been made on the electrical responses.

We have determined the recovery curve of the second electrical response as a function of the interval between the stimuli applied to the nerve, in a number of experiments. The curves we have obtained agree closely with those published by Lorente de Nó [1935] for the internal rectus muscle of the rabbit, excited by stimulation of the III nerve within the skull. A second response first appears when the stimuli are separated by 0.4 msec. Thereafter, the second response increases rapidly in size, and attains the size of the first in 4-5 msec. (Fig. 1).

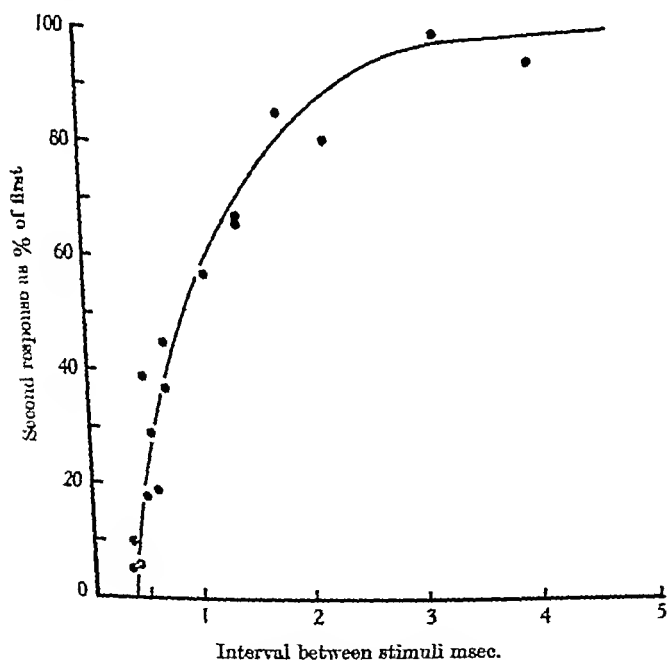


Fig. 1. Recovery of second electrical response of inferior oblique excited by two nerve volleys.

We have already given the reasons which led us to expect, in the eye muscle, a "two-volley" effect such as we have earlier described for avian muscle, and we have consequently examined our records carefully in search of it. In a number of instances, the second response has shown an

increase of from 5 to 8 % over the first response, when the single responses were regular, and there was nothing to suggest that the stimuli applied to the nerve were not maximal. It is, however, difficult to assess the significance of this difference. The second response of a pair is often shorter in duration than the first, especially if the muscle is below body temperature, and this alone might account for some of the apparent increase in size of the second response. A further difficulty is introduced by the presence in many muscles of the slow negative wave, which follows the action potential spike, and this itself, as we have suggested previously [Brown & Harvey, 1938a] is sufficient to obscure the true size of an action potential arising from it.

Effects of curarine

The extrinsic ocular muscles are affected by smaller doses of curarine than the muscles of the lower limb. Comparisons of the doses required to produce paralysis in the limb and eye muscles in different animals gave fairly clear evidence of this, but in one experiment we compared directly the effects of curarine on the two types of muscle. In a decerebrated cat of 2.6 kg. we recorded simultaneously the response of the inferior oblique muscle to stimulation of the III nerve in the orbit, and those of the tibialis anterior to stimulation of the peroneal nerve. Curarine was administered intravenously in an initial dose of 0.3 mg. followed by successive doses of 0.1 mg. each. The inferior oblique muscle showed a diminution of 50 % in twitch tension after the cat had received 0.5 mg., and neuromuscular transmission appeared to be almost completely interrupted by a total of 0.6 mg. Simultaneously, the twitch of the tibialis anterior showed no change in tension after 0.5 mg., and was reduced only 15 % after 0.6 mg.

Response to two nerve volleys after curarine

The eye muscle affords a very good preparation for the study of the effects of two nerve volleys after partial paralysis of neuro-muscular conduction by means of curarine. It has been shown previously [Brown, 1938] that the action potential of the tibialis anterior in response to the second of two closely following motor nerve volleys, after curarine, is greater than that given in response to the first. The same applies to the eye muscles, but the short duration of the action potential enables this phenomenon to be studied at much shorter time intervals than in the larger muscle. In each experiment, as a preliminary to the administration

of curarine, we took sufficient observations to enable us to plot a curve, relating the size of the second action potential as a percentage of the first, against the interval between the two nerve volleys, a "normal recovery curve" being thus obtained (cf. Fig. 1). Curarine was then administered in a dose sufficient to reduce the action potential of the muscle, in response to a single maximal nerve volley, to a fraction of its original value, and the size of the second response at various intervals was again determined. Subtraction of the figure for the normal recovery of the second response,

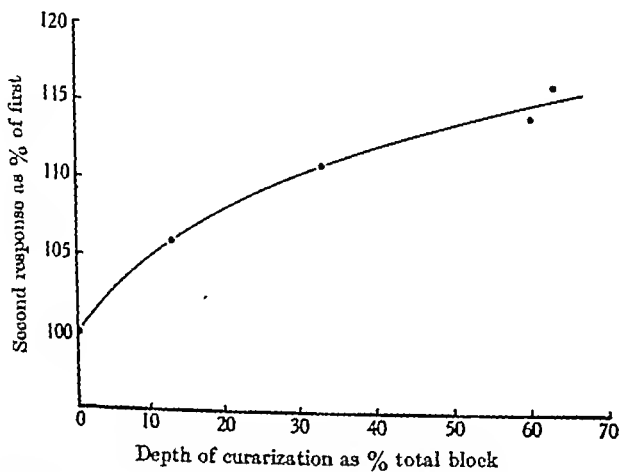


Fig. 2. Relation between depth of curarization and degree of excess response to second of two volleys 10 msec. apart.

at any given interval between the stimuli, from the figure for recovery at the same stimulus interval in the partially curarized preparation, enables one to determine how much the degree of recovery of the second response in the curarized preparation differs from the normal. We have made such determinations in a number of experiments at various depths of curarization.

The amount of "excess response" [cf. Katz, 1939] at any given interval between the stimuli is a function of the depth of curarization (Fig. 2). In Fig. 3 are shown curves of the excess response at a level of 67% curarization. The maximum appears, in most experiments, to lie between 0.8 and 1.5 msec., after which there is a steady decline to about 10 msec., where the excess response just disappears. At intervals between the absolute refractory period of the nerve and 1 msec., the results have been less regular and more difficult to repeat consistently, on account of the difficulties of measurement of the two closely superimposed action

potentials. In one experiment we observed an increase in the muscular action potential over the single response when two nerve volleys were set up at an interval less than the refractory period of the neuro-muscular system as previously determined.

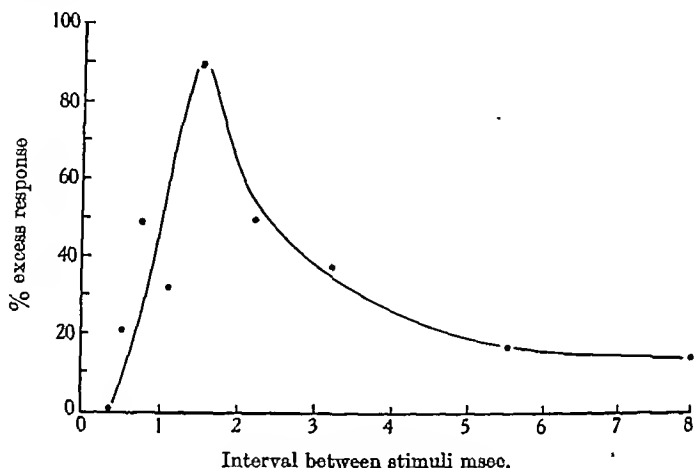


Fig. 3. Excess response to second of two nerve volleys in partially curarized muscle.

At intervals longer than 10 msec. the second response becomes smaller than the first, and, as in the tibialis muscle, does not recover its full size for some seconds (Fig. 4).

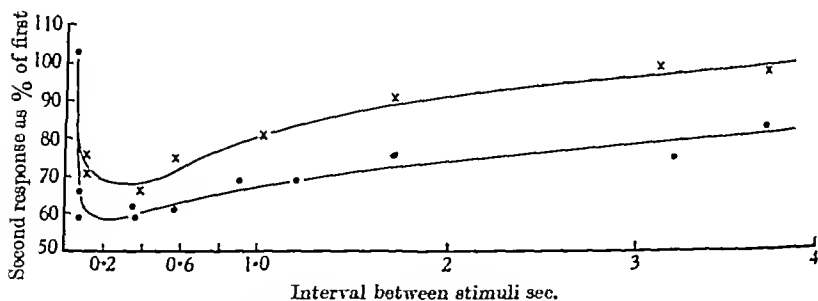


Fig. 4. Depression of response to second of two volleys at intervals greater than 20 msec. Upper curve (x), 75% curarized. Lower curve (•), 89% curarized.

We have been able to show that these peculiarities in the response of the muscle to nerve stimulation after curarine do not appear when the fully curarized muscle is stimulated directly (Fig. 5). Similar results were obtained on fowl muscle [Brown & Harvey, 1938a], but the eye muscle is better adapted for direct stimulation. A further point of interest is

revealed by the mechanical responses of the muscle to two nerve volleys set up close together in a suitably curarized preparation. The second mechanical response, like the electrical response, is considerably greater than the first, if the two nerve volleys are suitably timed. At longer intervals the second mechanical response, again like the electrical, is weakened and only recovers when the interval between the stimuli is

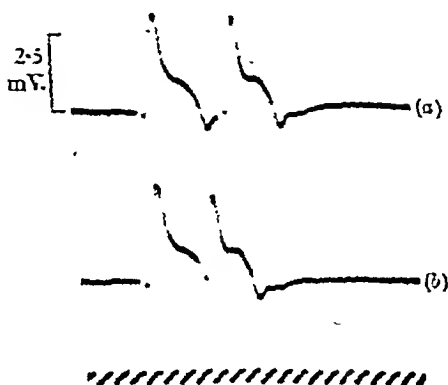


Fig. 5. Responses of fully curarized inferior oblique to two direct stimuli, (a) 5, and (b) 3.7 msec. apart. Time, msec.

much longer. When the preparation is curarized to such a degree that the twitch tension is reduced to 10% or less of the normal, the recovery of the second twitch tension may not be complete until 4 sec. after the first twitch.

Effects of eserine

The immediate effect of eserine, whether given intravenously in the usual doses, or in a dose of 0.5 mg. by intracarotid injection, is the production of twitching in the muscle. These "spontaneous" twitches, in the case of the eye muscle, are sufficiently synchronized to produce a tension equal to or even greater than that of a maximal nerve twitch before eserine (Fig. 6). They are accompanied by brief outbursts of action potentials which, although of smaller magnitude, resemble closely the potentials following a single nerve volley, after eserine. The twitching is maximal a few minutes after the administration of eserine, and gradually wanes. It is increased by stimulation of the nerve.

Single nerve volleys evoke, after eserine, a very striking repetitive discharge in the eye muscle. As might be expected from the intensity of the electrical effects, the potentiation of the twitch tension by eserine is very striking (Fig. 10). In one experiment, for instance, in which the maximal indirect twitch tension was 0.2 g. before eserine, the administration of 0.5 mg. eserine by carotid injection caused it to increase to 3.3 g.—a more than sixteenfold increase.

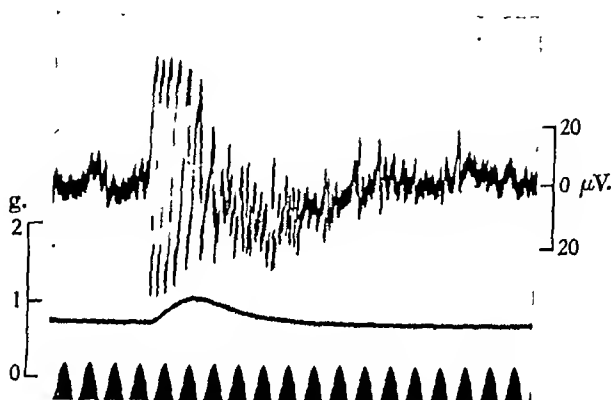


Fig. 6. Cat. 3.8 kg. myogram and action potential (belly-tendon) of inferior oblique, showing spontaneous contraction after intracarotid injection of 0.3 mg. eserine.

The electrical record of the response of the muscle under eserine to single nerve volleys shows that the initial spike potential is followed by a regular series of spikes which undergo a logarithmic decrement (Figs. 7, 10). As many as ten perfectly discrete potentials may follow a single nerve volley.

One very characteristic feature of the response of tibialis anterior after a full dose of eserine is that the repetitive discharge resulting from a single nerve volley is not increased in amplitude or duration by a second nerve volley falling within the period of synchronized repetitive response [Brown, 1937]. This does not hold for the eye muscle, in which we have specifically investigated this point. A cat of 2.3 kg. body weight, which had already received 0.7 mg. eserine intravenously, was given a further dose of 2 mg. after an interval of 1 hr. 45 min. These doses are amply sufficient to produce a maximal potentiation in the tibialis and gastrocnemius muscles. Within 15 min. after the second dose of eserine, the electrical responses of the eye muscle to single and double nerve volleys were recorded (see Fig. 7). The results are shown graphically in Fig. 8.

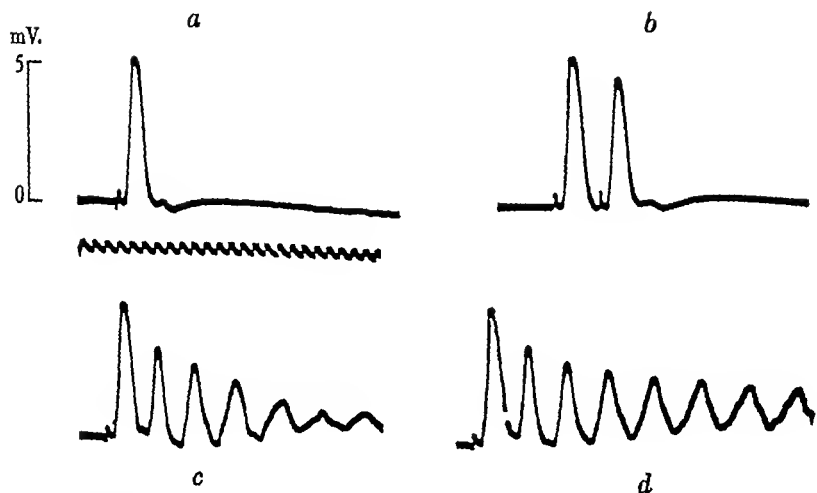


Fig 7 Action potentials of inferior oblique, before eserine: (a) single nerve volley, (b) two volleys 3.4 msec. apart; after eserine: (c) single volley, (d) two volleys 2.4 msec. apart. Time, msec. For further details see text.

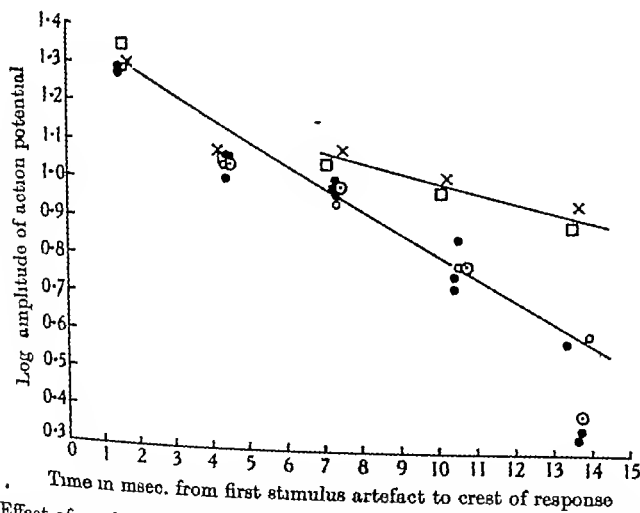


Fig 8 Effect of single and double nerve volleys on action potentials of inferior oblique after a full dose of eserine (•) single volleys, (○) two volleys 0.5 msec. apart, (⊙) 0.7 msec. apart; (□) 1.1 msec. apart, and (×) 1.4 msec. apart

The logarithms of the amplitudes of the first five successive responses have been plotted against the interval at which each response succeeds the initial stimulus artefact. It is clear that when the pairs of stimuli are given at intervals of 0.5 and 0.7 msec., they produce no more effect than the single stimuli, whereas, with pairs of stimuli at 1.1 and 1.4 msec., the third and successive deflexions in a repetitive series are of greater amplitude, and occur a little earlier in the series, than those following a single stimulus. In this particular experiment, measurements made before the administration of eserine showed that a second response was easily visible when the nerve volleys were 0.5 msec. apart.

We have made some measurements of the amplitudes of the second responses in series following single nerve volleys under eserine, which reveal some points of interest. In the normal muscle (without eserine), excited by two nerve volleys, the second of the two resulting responses attains its crest at the same time after the first response, irrespective of the interval between the stimuli, when this is less than 1.2 msec. This effect is shown graphically in Fig. 9, in which the amplitude of the second response is plotted against the interval between its crest and the artefact of the first stimulus. This shows that no second response, however early its stimulus, can occur less than 3.5 msec. after the first stimulus [cf. Lucas, 1910]. Measurements of the responses of a muscle under eserine show that the second response of the repetitive series is regularly about 60 % of the first response, and that its crest appears 4.2–4.5 msec. after the first stimulus artefact. If these figures are plotted on the curve for two nerve volleys in normal muscle (Fig. 9), it is seen that the second action potentials in the repetitive series evoked in the eserinated muscle by a single nerve volley lie at a point 0.7–1 msec. later than they would occur if they were the responses of a normal muscle to a second nerve volley. Their position on the curve could be accounted for if they were some 20 % submaximal, but they appear to be either maximal, or very nearly so, since second nerve volleys at intervals of about 2 msec. after the first do not significantly increase the magnitude of the second response. Such volleys effectively reach the muscle, as shown by their effect on the third and subsequent responses, and, in normal muscle, are followed by responses 15–20 % greater than the second response after eserine. It would appear, therefore, that the second response of the repetitive series evoked by a single nerve volley in the eserinated muscle is a maximal response, and that it cannot be advanced in time by a nerve volley which is capable of affecting subsequent responses. The simplest explanation of this phenomenon is that the refractory period of the muscle fibre has

been prolonged by the eserine. Although the second, maximal response of the repetitive series cannot be affected by a second nerve volley more than 1 msec. after the first, the effect of such a volley persists and augments the third, and subsequent responses. The possible prolongation

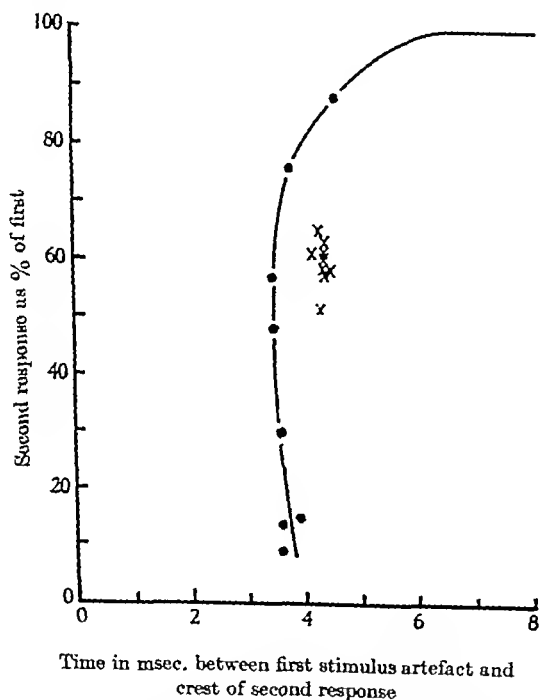


Fig. 9. Relation between amplitude and time of occurrence of response to second of two volleys in normal muscle (---). The points marked (x) show the second responses of the repetitive series following single and double volleys in muscle under eserine.

of the refractory period of the muscle fibre does not, however, explain the failure of the volleys at 0.5 and 0.7 msec. to affect the third and subsequent responses. Either eserine has increased, and indeed almost doubled the refractory period of the nerve, or some other factor is involved.

Effect of tetanic stimulation after eserine

The response of a muscle fully poisoned with eserine to tetanic stimulation of its nerve is, characteristically, little different from the response to a single nerve volley, and this phenomenon is very evident in the eve muscle (Fig. 10). This figure has an additional interest, in that,

as will be seen, the frequency of the stimulation used happened to be almost exactly that of the natural rhythm of the repetitive response of the eserinizied muscle following a single shock to the nerve. The single nerve volley evoked a response from the muscle of 3.3 g. tension, and the maximum attained by the full tetanic stimulation at 220 per sec. was only 3.5 g. It is obvious that the additional effect of volleys subsequent to the first one, although visible in both the electrical and mechanical responses, is very small. After the preliminary brisk contraction the tension declines, and is maintained at a reduced but constant level

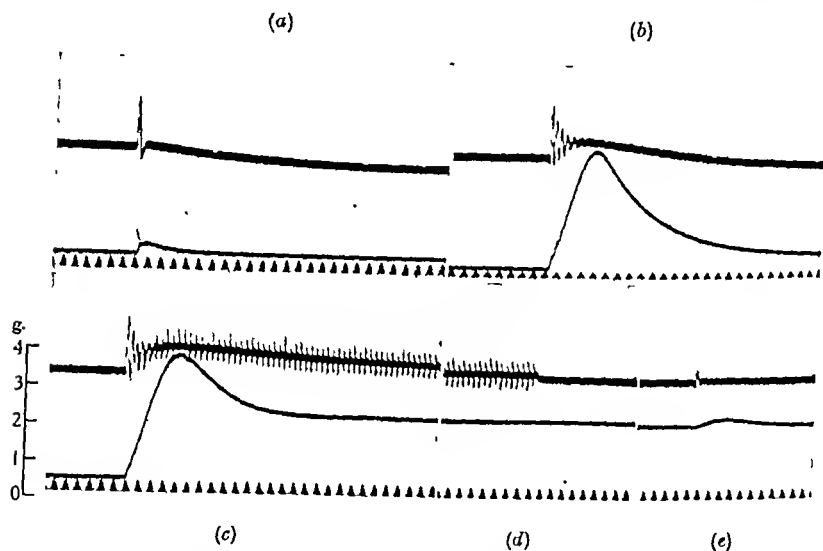


Fig. 10. Myogram and action potential (belly tendon) of inferior oblique. Response to single maximal nerve volley (a) before and (b) after eserine. (c) Response after eserine to maximal stimulation of nerve at 220 per sec. Between (c) and (d) 0.25 sec. has elapsed. (e) Response to single maximal nerve volley 0.4 sec. after end of tetanic stimulation. Time 10 msec.

throughout the continued stimulation. This tension may, under certain conditions, outlast the cessation of the stimulus and, indeed, may actually persist for some minutes (Fig. 10). The conditions under which this prolonged response is seen are that the animal should have received a full dose of eserine, and that the frequency of stimulation should be in the region of the fusion frequency. Single stimuli applied to the nerve during this after-tension evoke a "twitch" which is very much less in tension than the normally potentiated "twitch", elicited before the tetanus. Although the "twitch" is much reduced in tension, until it may be even

less than the pre-eserine twitch, the action potential accompanying it is still repetitive.

It was obviously of considerable interest to obtain some information about the occurrence, or otherwise, of action potentials during the prolonged after-tension following a tetanus, since the reduction of the "twitch" tension strongly suggested that the persistent tension was due to a contracture, and not to propagated contractions throughout the muscle. Belly-tendon records show that there is considerable electrical activity during the period of maintained after-tension, and there is certainly little suggestion of the abrupt and long-lasting electrical silence which accompanies the development of a true contracture, such as that evoked by acetylcholine in denervated mammalian muscle. The reduction in twitch tension however is, as pointed out above, strongly suggestive of the existence in some fibres, at least, of a contracture proper. If reference is made to Fig. 11, it will be seen that a contraction evoked by the injection of acetylcholine was of a tension in excess of the maintained post-tetanic contraction, and yet twitches superimposed on this were not in any way reduced. The conclusion must be, then, that there is a large element of contracture in the maintained tension which follows a tetanization of the eserinated muscle through its nerve.

Effect of eserine on curarized muscle

There is one other action of eserine which we have been able to study in this muscle. We gave a decerebrated cat a dose of curarine sufficient to block neuro-muscular transmission to the eye muscle permanently and completely. We then excited the muscle by directly applied shocks, making alternate shocks maximal and submaximal. The administration of eserine in a dose of 0.5 mg. by the lingual artery resulted in an increase in the response to the submaximal stimuli, without any change in the response to maximal stimuli. Renewed application of the shocks to the nerve showed that the eserine had not restored the transmission blocked by curarine. Eserine, accordingly, lowers the threshold of the muscle fibre to direct electrical excitation, apart from its more dramatic effects on neuro-muscular transmission.

Effects of acetylcholine

The special sensitivity of the extrinsic ocular muscles to acetylcholine has been known for some years [Duke Elder & Duke Elder, 1930], and we have been able to confirm it. We have, in addition, obtained some evidence of the nature of the response. Most of our injections have been

made into the carotid, through the central stump of the lingual artery, as this provides a reasonably close access to the muscle for the injected solution, and gives more reliable and consistent results than injection by the intravenous route, though it is not completely comparable with the close arterial method we have used for certain leg muscles. We have made no attempt to determine threshold doses by any route of injection, as so many factors tend to vitiate the results. With doses of 0.5–1 mg. intravenously, and 50–100 μ g. arterially, the eye muscle goes into a prolonged contraction of considerable tension. This contraction is accompanied throughout its course by oscillatory action potentials (Fig. 11*a*). Twitches



Fig. 11. Myogram and action potentials (concentric needle) of inferior oblique. (*a*) Response to carotid injection of 100 μ g. acetylcholine. (*b*) Effect of carotid injection of 50 μ g. acetylcholine during excitation of nerve with single maximal shocks. Time 0.5 sec.

evoked by nerve stimulation are not reduced either in tension or action potential by this response to acetylcholine (Fig. 11*b*). We are convinced, therefore, that normally the eye muscle responds to acetylcholine with a tetanic contraction and not with a contracture. After eserine, however, there is some evidence that an element of contracture enters into the response. A large dose of acetylcholine, e.g. 100 μ g., then caused a big mechanical response which was accompanied, at first, by oscillatory potentials. These, however, rapidly subsided, and the tension was maintained with electrical "silence" of the muscle.

In the denervated muscle the element of contracture is much more in evidence. Any response of more than 3 g. in tension has no accompanying action potentials after the initial rise of tension (Fig. 12). We made some

observations on the least doses of acetylcholine required to cause a recordable contraction of the denervated eye muscle, and found that it was not exceptionally sensitive. Quite clear contractions were evoked by doses less than $0.5 \mu\text{g.}$ given arterially, but at least $10 \mu\text{g.}$ were required to evoke an unequivocal response by intravenous administration. As might be expected, from the distance the acetylcholine had to travel in the blood after any of these injections, its effects were materially potentiated by the administration of eserine. The use of the eye muscle gave us an opportunity of testing another point. Rosenblueth & Lucco

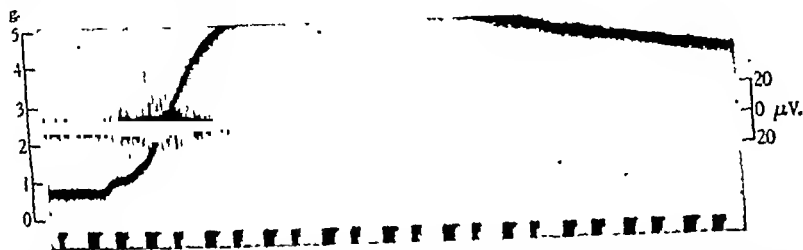


Fig. 12. Myogram and action potential (belly tendon) of inferior oblique 7 days after nerve section. Spontaneous discharge and response to $25 \mu\text{g.}$ acetylcholine by carotid injection. Time 0.2 sec.

[1938] have claimed that eserine increases the spontaneous electrical activity of a denervated muscle. In previous investigations, this activity has been tested by means of concentric needle electrodes which, although admirable for revealing the existence of asynchronous activity in a muscle, are not well adapted for quantitative comparisons, since the least movement is liable to displace them from the fibre group with which they are in contact. The small size and regular design of the eye muscle has allowed us to record such potentials by leads from belly to tendon, and with this method the activity of the whole muscle can be detected. Under these conditions, we were unable to detect any effect on the spontaneous activity of the denervated eye muscle when 0.5 mg. of eserine was injected into the carotid artery.

DISCUSSION

The extrinsic ocular muscles provide a very suitable preparation for the study of many problems of muscle physiology. Their small size and uniformity, and the fact that their fibres run the full length of the muscle, give them obvious advantages, especially for making and interpreting electrical records. The very small temporal dispersion in the twitch

response makes the gross action potential of the muscle nearly as useful as that of a single fibre. The reason for the small temporal dispersion is not far to seek: these muscles are composed of relatively few fibres, and these few fibres are supplied by relatively many nerve fibres; that is, the innervation ratio is very small. Tergast [1873] has stated that the ratio of nerve fibres to muscle fibres may be as low as 3 in these muscles, but he apparently took no account of the possibility that sensory fibres may be present. In any case, it is probable that not more than 10 muscle fibres are supplied by a single nerve fibre. This low innervation ratio, and the apparent homogeneity of the muscle fibres in their physiological properties, have other effects. We have shown that the eye muscle appears to have a greater sensitivity to curarine than other muscles. This may be due to a greater mean sensitivity of the fibres; it may, however, be rather an expression of a much greater uniformity in threshold and sensitiveness of the fibres of the eye muscle than that found in muscles with many more fibres, such as the tibialis. In the eye muscle, with all the fibres having a nearly identical excitability to the effect of a nerve impulse, a relatively small depression of that excitability would exclude a very large number of the fibres, or even all of them, from response to a nerve volley, whereas in a muscle with a wider scatter of the excitabilities of individual fibres, a large change in excitability would involve proportionately fewer fibres, and a wide shift of mean threshold may be needed to complete paralysis.

The phenomena occurring in eye muscles with less than paralysing doses of curarine are of considerable interest. They confirm the previous observations on the tibialis muscle so treated, in which it was shown that a partially effective nerve volley left behind it a brief period of raised excitability of the muscle fibres to a subsequent testing volley, followed by a period of depressed response of much greater duration. The rapidity of the action potential of the eye muscle has enabled us to obtain information about the early phases of the facilitation phenomenon, in the period during and immediately after the recovery of the nerve from the passage of the first impulse. The curves obtained are, in general form, very similar to those of Bremer & Kleyntjens [1937] for frog's muscle, but are, of course, on a much faster time scale. The long period of depression, which follows the period of excess response, is even more evident in the eye muscle than in the tibialis anterior. In a previous paper [Brown, 1938] it was suggested that this long depression was due to insufficiently rapid replacement of acetylcholine at the nerve endings after the passage of the first impulse. Subsequent experiments have given no evidence

definitely in favour of, or against, this suggestion. The absence of the effect in normal avian muscle which, in other respects, behaves very much like a mammalian muscle under a small dose of curarine, suggests that the long period of depression may be a property introduced by that drug, and not an expression of the normal excitability cycle of the neuromuscular system.

The effects of eserine on the eye muscle are, in general, the same as on other mammalian muscles, but the shape and size of the eye muscle have enabled them to be analysed in a little greater detail. There is some evidence from our experiments that eserine may evoke a small prolongation of the refractory period of the end-plate-muscle system. The failure of a second nerve volley, at intervals up to 0.7 msec., to affect the decline of the repetitive responses of the fully eserinizied muscle to the first nerve volley may mean either an approximate doubling of the absolute refractory period of the nerve, or that the impulse set up at these short intervals, and travelling only a short distance along incompletely recovered nerve, must arrive at the nerve terminals before it has attained full magnitude, and there liberate an amount of acetylcholine insufficient to affect the subsequent responses. The possibility that the refractory period of nerve is lengthened by eserine is directly contrary to the findings of Bremer & Kleyntjens [1937] who concluded that eserine shortened the refractory period in the frog, but the complexity of the effects of eserine on mammalian muscle makes difficult any deductions based on analogy. The recent description by Masland & Wigton [1940] of impulses of peripheral origin in motor nerves after eserine and prostigmin adds to the difficulty of interpretation of the effects of centrifugal impulses on the eserinizied muscle. It is, indeed, possible that the failure of volleys at 0.5 and 0.7 msec., which we have described, may be accounted for by the interference in the nerve of the artificially evoked centrifugal volley with the first of the centripetal impulses arising from the nerve endings in the contracting muscle.

There is an apparent discrepancy between the effects of multiple excitation of the motor nerve of the eye muscle under eserine, and those previously recorded for the tibialis anterior [Brown, 1937], in which it was found that, under eserine, a second volley, set up during the synchronized repetitive discharge produced by the first, failed to enhance this discharge or materially to affect the tension developed by the muscle. The experiment recorded in Fig. 10, however, shows that although nerve volleys after the first may evoke some change in the rhythmic series of action potentials following the first nerve volley, their whole effect upon

the tension developed by the muscle is trivial. The reason for their failure to affect the total tension is revealed by the persistence after the tetanic stimulation has stopped, of a muscle shortening which seriously depresses the response of the muscle to test shocks applied to the nerve, in other words, the development of a contracture. We have similarly obtained evidence that there is some element of contracture in the response of the eserinizied eye muscle to acetylcholine. Previous work on denervated mammalian, on avian and on amphibian muscle had suggested that the depressant effect of acetylcholine and of nerve stimulation after eserine might be attributable to the development in the muscle fibres of a contracture, effectively blocking the initiation and propagation of normal excitation along the muscle fibre. These are the first, and only instances in which such a contracture has been observed in a normally innervated mammalian muscle, and we do not suggest that a fully developed contracture occurs in other muscles of the mammal. It appears probable, however, that an analogous block of propagation in the muscle fibre resulting from a local depolarization may account for the depressant effects of accumulating acetylcholine, whether artificially applied or generated by nerve stimulation, in the fully eserinizied muscle.

SUMMARY

1. The responses to nerve stimulation of the extrinsic ocular muscles have been studied in decerebrated cats. The small size and uniform structure of these muscles make them particularly useful for electrical recording.

2. The refractory period of the nerve-muscle preparation is about 0.5 msec., and recovery of the second response is complete in 4-5 msec.

3. The rapidity of the action potential has enabled information to be obtained about the early stages of the facilitation phenomenon, following a nerve volley, which is revealed by treatment with less than paralyzing doses of curarine. The increase in the response of the muscle to the second of a pair of maximal nerve volleys reaches its maximum between 0.8 and 1.5 msec., and thereafter declines, to disappear at a stimulus interval of 10 msec.

4. The period of excess response is followed by a period of depression of the second response, both electrical and mechanical, which may last as long as 4 sec. The facilitation and depression are not observed in the fully curarized muscle, stimulated directly.

5. After eserine, single nerve volleys evoke a regular series of spikes which undergo a logarithmic decrement. Double nerve volleys have complex effects, the analysis of which suggests that eserine may prolong the refractory period of the muscle, and interfere with the conduction, in the nerve, of the second of two closely succeeding volleys.

Eserine lowers the threshold of the fully curarized muscle to direct electrical excitation.

6. Acetylcholine, injected into the carotid artery, or into a vein, evokes a contraction of long duration. The contraction is accompanied throughout its course by oscillatory action potentials.

7. After eserine, however, both acetylcholine and repetitive nerve stimulation evoke a contracture, which blocks the propagation of excitation along the muscle fibre.

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VOLUME OF INTERFIBRE SPACES IN FROG MUSCLE AND THE CALCULATION OF CONCENTRATIONS IN THE FIBRE WATER

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THE methods hitherto used for determining the volume of the interfibre spaces in muscle have been mostly of three kinds; firstly, the histological method, from which exact results cannot be expected; secondly, that depending on the ratio of muscle chloride to the chloride in the external fluid, the muscle membrane being assumed to be impermeable to anions; and thirdly, measurements based on the fall of conductivity after washing in isotonic glucose or sucrose solution, electrolytes being considered to diffuse only from the interfibre spaces.

It is true that for muscle *in vivo* the chloride ratio may give a measure of the interfibre spaces which is in excess of the real value by only about 1-3% of the total muscle volume. For frog's sartorius the mean figure so obtained is 14-15 ml./100 g. muscle using the data of Fenn, Cobb & Marsh [1934] for the plasma chloride, and of Conway & Kane [1934] and of Fenn *et al.* [1934] for the muscle chloride. For mammalian muscle mean figures of 14 ml. have been given [e.g. Winter, 1934] or of 12 ml. as obtained here, whereas the inulin method gives only 7 ml. [Conway & Fitzgerald, 1940] and the use of radio-sodium, 8.5 ml. [Hahn, Hevesey & Rebbe, 1939]. This latter figure was raised to 11 ml. on excluding the fat and other extraneous tissue.

The use of the chloride ratio for muscle immersed in Ringer's solution for over 15 min. gives very erroneous values for the interfibre volume. The mean figure obtained is then 26 ml./100 g., whereas the true interfibre volume is in the region of 9-13 ml./100 g., as shown by the methods described here. The high figure for the chloride ratio with immersed muscle has led to the use of the term 'chloride spaces' with a different

meaning than pure interfibre volume [e.g. Ghaffar, 1935; Eggleton, Eggleton & Hamilton, 1937], the difference being referred to chloride adsorbed on the surface of the fibres [Eggleton *et al.* 1937].

With regard to the conductivity measurements, Schulze's calculations [as corrected by Fenn, 1936] amount to 13.9 ml./100 g. This is likewise somewhat too high, since electrolytes other than those in the interfibre spaces or adsorbed on the fibres are lost while soaking in isotonic sugar.

We have used here four different methods, three of which may be described as direct and one indirect. In the first method inulin was used instead of chloride to measure the interfibre spaces. Inulin penetrates into the tissue spaces and the ratio of muscle inulin to external inulin will apparently give for these a maximum value. In the second method magnesium was used, since it only very slowly penetrates into the excised muscle fibres. The magnesium already inside is quite indiffusible over short periods and loses only about 10% of its value after soaking for 24 hr. in magnesium-free Ringer solution at 2-3° C. [Conway & Cruess-Callaghan, 1935]. In the third method the diffusion of muscle sodium into isotonic glucose was studied and the amount in the interfibre spaces measured from the diffusion curve. Lastly, the amount within the fibres of normal muscle was calculated on the basis of a Donnan equilibrium—a procedure which will be shown later to be fully justified. This figure, amounting to only 1.1% of the external, is subtracted from the total muscle chloride. We have also made some studies of the total circulatory space in the fresh excised sartorius of the frog, showing that its mean value is approximately 2.3 ml./100 g. tissue.

ANALYTICAL METHODS

Sodium. This was estimated by a modification of the method described by Salit [1932] modified for use with the Pulfrich photometer. 1 ml. samples of the ash extract were used instead of the 2 ml. described and the other reagents added in corresponding volume. In the final comparison 10 ml. were taken, 0.25 ml. of the potassium ferrocyanide solution added, and, after standing 5 min., the reading taken in the Pulfrich photometer. The following equation was used (with Filter S 50):

$$\text{Mg. sodium in 1 ml. solution} = 0.115 (E - e),$$

where E is the extinction with 0.5 cm. stratum layer, and e that of the blank. This relation was found to hold best for solutions having extinctions between 0.3 and 1.4. With extinctions greater than 1.4, diluting before developing the colour was carried out, as described by Salit.

Potassium. This was determined by the method of Shohl & Bennett [1928] with preliminary ashing as described by Fenn & Cobb [1934]. The final coloured solution of the potassium iodoplatinate was examined spectrophotometrically, using a 0.5 cm. stratum layer and Filter S 50 in the Pulfrich photometer. The potassium in the sample solution of the ash was calculated from the following equation (applying to standard solutions treated exactly as the muscle):

$$\text{Mg. potassium} = 0.32 (E - e),$$

where E is the extinction of the potassium iodoplatinate and e the extinction of a blank, prepared under the same conditions.

Chloride was measured by a micro-diffusion method [Conway, 1935].

Inulin. This was determined after varying times in the 1.3% sodium sulphate solution in which the muscles were suspended after previous soaking in an inulin-Barkan fluid. The inulin was oxidized completely by potassium permanganate and sulphuric acid and the carbon dioxide measured by a micro-diffusion method [Conway, 1939]. In the procedure, 2 ml. of the solution were taken in the outer chamber, some solid permanganate added and 1 ml. of 70% sulphuric acid. The inner chamber contained 1.3 ml. of $N/40$ $\text{Ba}(\text{OH})_2$ and 1 ml. was removed at the end into a small tube, a drop of thymolphthalein solution in alcohol added and the mixture titrated with $N/100$ HCl .

The inulin-Barkan solution in which the muscles were soaked initially was diluted 200 times and the inulin determined in a similar way. It was necessary to express concentrations only in terms of this fluid.

To measure the oxidizable non-inulin material diffusing from muscle, control curves were obtained with companion tissues similarly treated, but with inulin absent from the original soaking fluid.

EXPERIMENTAL METHODS AND RESULTS

The inulin method

Twenty sartorii, of about 50 mg. mean weight, were soaked for 2 hr. at room temperature in 50 ml. of an inulin-Barkan solution with oxygen (97%) and carbon dioxide (3%) bubbling. The solution contained 2% inulin and 0.65% NaCl , the remaining constituents being the same as described by Barkan, Broemser & Hahn [1921]. The muscles were then removed, dried quickly, weighed and transferred to 30 ml. of a 1.3% sodium sulphate solution. In this way they were stirred by bubbling with oxygen for some hours. Throughout, at 4, 9, 16, 25, 49 and 100 min., 10 ml. samples were removed and replaced by 10 ml. of fresh sulphate

solution. With each of these samples three or four analyses of 2 ml. were carried out as described under 'Analytical Methods'. Control groups of muscles were treated in a similar way, but without inulin in the soaking fluid. With these the oxidizable non-inulin substances diffusing into the sulphate was determined.

Four groups of such experiments were carried out, the mean weight change on soaking being a loss of 1.6%. The results are summarized in the curve through the mean values in Fig. 1. It will be seen that about 2 hr. are sufficient for the outward diffusion, and by inference for the diffusion inwards.

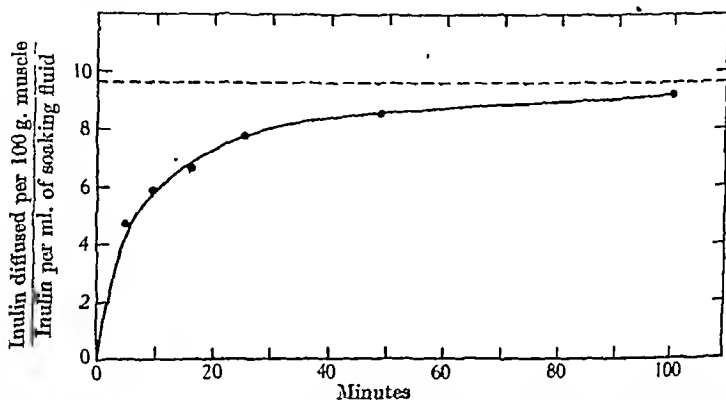


Fig. 1. Volume of the interfibre spaces in the sartorius muscle as measured by inulin diffusion into sodium sulphate (1.3%) after preliminary soaking for 2 hr. in an inulin-Ringer solution. Room temperature.

The ratio of muscle inulin (as judged from the inulin recovered after 100 min.) to the external inulin indicates a mean value for the interspaces of 9.4 ml./100 g. The slightly higher value of 9.6 ml./100 g. could be taken if recoveries after a hypothetically very long time were considered.

The four individual experiments gave 10.3, 8.5, 7.5 and 13.0 ml./100 g. respectively. The oxidizable material diffused from the control muscles amounted to approximately 1.07×10^{-3} m.equiv. carbon dioxide per g. per min. In 60 min., when the inulin diffusion from the soaked muscles was almost complete, this non-inulin oxidizable material accounted for about one-third of the total carbon dioxide found on oxidation.

The magnesium method

Chloride or sulphate ions diffuse almost completely into or out of the excised sartorius muscle immersed for 15 min. in Ringer or modified Ringer solutions. From a comparison of the diffusion coefficients of

magnesium chloride or sulphate with sodium chloride or sulphate the diffusion of the magnesium into or out of the interfibre spaces should likewise be almost complete in less than 30 min. At the same time the membrane in excised muscle is not quite impermeable to magnesium ions, but the penetration is comparatively very slow. The magnesium already contained in the muscle (24.7 mg./100 g.) is practically indiffusible and presumably organically bound within the fibre [Conway & Cruess-Callaghan, 1937]. When the sartorius muscle is immersed therefore in modified Ringer fluid containing 200 mg. magnesium/100 ml. (replacing an equivalent amount of sodium) the interfibre volume should be clearly

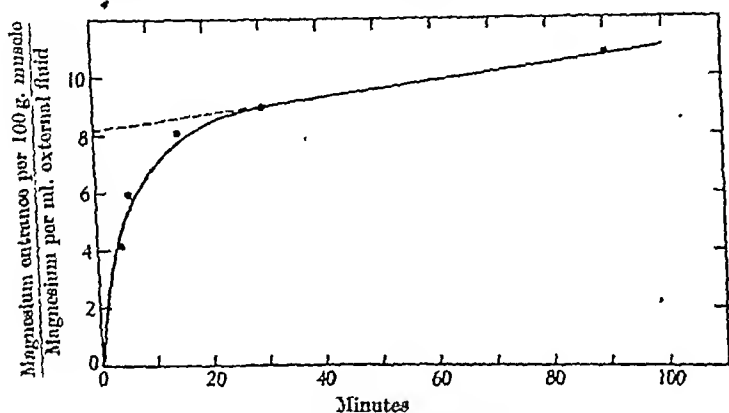


Fig. 2. Volume of interfibre spaces as measured by magnesium entrance from a magnesium-Ringer solution, containing 200 mg. magnesium/100 ml. and 0.26% NaCl. Room temperature.

determinable from the curve of magnesium increase expressed as a percentage of the external substance. Fig. 2 gives a summary of the results obtained for the magnesium increase after immersion. Each dot represents the mean change of magnesium for two or three experiments in each of which four muscles from the same number of frogs were used. The magnesium originally present was determined from companion muscles directly analysed. The ordinates give the increase per 100 g. original tissue divided by the magnesium content in 1 ml. of external fluid. From the line of slow increase after 30 min. produced backwards to cut the ordinate from the origin the interfibre spaces have a volume of 8.2 ml./100 g. tissue. This is similar to the inulin figure of 9.6 ml./100 g., and the difference may be attributed largely to the mean increase in weight of 5.8% in the magnesium immersions compared with a loss of 1.6% with inulin. That such weight changes can affect the interspace

volume is shown by the fact that using the magnesium method in a further series of experiments in which the external fluid (of higher tonicity than before) caused a fall in weight of 12·5%, the interfibre space was found to be 12·6 instead of 8·2 ml./100 g. We could conclude therefore that a relative *fall* in weight corresponding to 18·3 ml./100 g. of original tissue appears to be associated with an *increase* in the interfibre spaces of 4·4 ml./100 g. of fresh muscle. If we were to use this relation in a proportionate way the space would be 9·2 ml. with the inulin method and 9·6 ml. with the magnesium method, when no change in weight occurred on immersion.

The following methods enable us to assess the total sodium chloride outside the fibre, and to calculate the chloride inside the membrane.

The sodium and chloride method

In a series of experiments observations were made on the diffusion of sodium, potassium and chloride from sartorii immersed in glucose solution (3·2–3·8%). Urano [1908] seems to be the first to have made systematic observations on the inorganic cation and anion changes in muscle after immersion in isotonic sugar. It was shown that the muscle is free of sodium and chloride after several hours immersion, whereas potassium, though lessened, is still largely retained. Fahr [1908], confirming these findings, drew the same conclusions as Urano that not only chloride but also sodium is contained between the fibres and not within them. Later, Mond & Netter [1932], perfusing a frog's hind limbs with isotonic glucose for short periods (4–6 min.), showed that the sodium came out in two phases and that only part of it could be considered to exist free between the fibres, concluding from further experiments that the sodium was bound in some way in the fibre membrane itself. That part of the sodium behaves differently from the chloride is at once evident from exact analyses of sodium and chloride mean values in frog's plasma and muscle [e.g. Fenn *et al.* 1934]. The mean ratio of muscle chloride (g./100 g.) to plasma chloride (g./100 g.) is 0·14, whereas the corresponding ratio for sodium is 0·22. Leaving for subsequent consideration the question of the localization of the extra sodium, our object here is to demonstrate the total sodium chloride external to the fibre by an exact construction of the time curve of emergence of sodium and chloride from the excised sartorius into isotonic glucose. In the procedure twenty sartorii were immersed in 20 ml. glucose solution (3·2–3·8%), stirred with oxygen and 2 ml. samples of the fluid removed at intervals up to 2 hr., being replaced each time by 2 ml. of fresh glucose solution. At the end the remaining

sodium in the muscle was determined. A similar procedure was used for potassium, except that 5 ml. was removed instead of 2 ml. as for sodium.

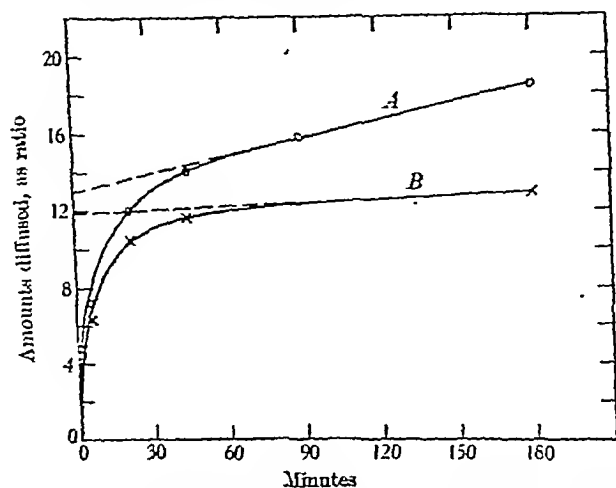


Fig. 3. Sodium (A) and chloride (B) diffusion from twenty sartorii muscles immersed in isotonic glucose, expressed per 100 g. muscle divided by the mean quantities in 1 g. plasma.

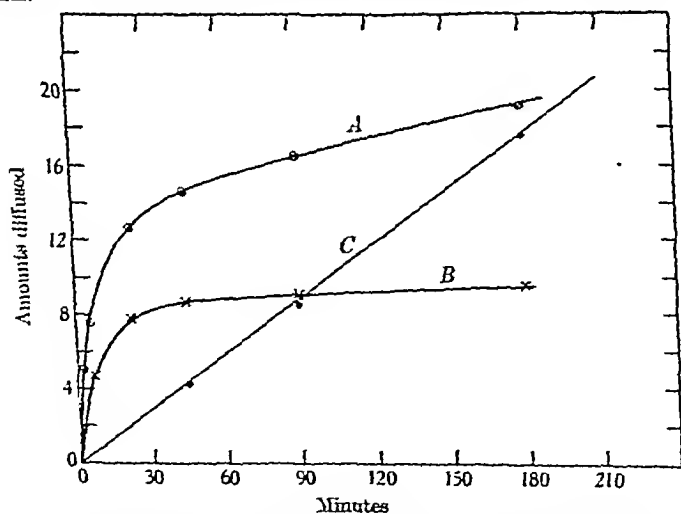


Fig. 4. Sodium (A), chloride (B) and potassium (C) diffusion from twenty sartorii immersed in isotonic glucose, expressed as m.equiv./kg. of original muscle.

For chloride a different procedure was adopted. Duplicate experiments were carried out in which four muscles were immersed in glucose solution for a certain interval, then removed and analysed for chloride. Fig. 3

gives a summary of the sodium and chloride results expressed as g./100 g. muscle divided by the mean concentrations in plasma (0.264 g./100 g. for chloride and 0.238 g. sodium/100 g. plasma [Fenn, 1936]).

Fig. 4 shows the actual quantities of sodium, potassium and chloride diffused and expressed as m.equiv./kg. of the original tissue. It will be seen that sodium comes out rapidly at first, after which it diffuses slowly and rather linearly so that an approximation to the amount in the first stage is given by extending backwards the line of the secondary increase to zero time. This gives 13%, and a similar extension of the chloride line gives approximately 12%. About 12.5 ml. would therefore represent maximum value for the interfibre space from such results. There is however, a total of 14% to be accounted for with chloride, leaving 1-2% as possibly present within the fibre. In the following method it will be seen that this is all the chloride that need be theoretically expected if the membrane is permeable both to chloride and potassium.

Indirect method from Donnan equilibrium calculations

That such calculations are relevant to this problem will be fully demonstrated in a later paper. They are based on the equivalence of the products of the potassium and chloride ion concentrations inside and outside the fibres. From the mean quantities of potassium, chloride and water in the muscle and plasma of the frog we have for the equivalence of the products

$$\frac{84.6 - 2.5s}{0.80 - 0.95s} \times \frac{10.5 - 74.3s}{0.80 - 0.95s} = \frac{74.3 \times 2.5}{0.954},$$

where 84.6 and 2.5 are the mean concentrations of potassium in muscle and plasma (m.equiv./kg.), 10.5 and 74.3 being the corresponding chloride values; s is the interspace plasma in kg., and 0.954 the water in kg. of plasma. (The sources of these data are given in a subsequent communication.)

From the equation s may be reckoned as 0.127, or in 100 g. muscle there are apparently 12.7 g. plasma and in 1 kg. of muscle 0.127×74.3 or 9.4 m.equiv. chloride. The total mean value for the muscle chloride is 0.5, so that only 1.1 m.equiv./kg. muscle (or 1.5/kg. fibre water) need be present within the fibre. No weight therefore need be attached to the resumed lack of chloride within the fibres depending on the apparent equivalence of chloride in whole muscle and that in the interfibre spaces, since the theoretical amount is too small for quantitative recognition by the methods used, and in particular the histological or histochemical methods.

The circulatory space in excised sartorii

In this determination the total haemoglobin in the sartorii was extracted and measured photometrically after conversion to alkali haematin and compared with blood suitably diluted and treated with alkali. The mean value for the circulatory space so obtained is naturally too high, as muscle haemoglobin is extracted as well as blood haemoglobin, but is of interest as a maximum figure. At the same time we have made some measurements of the extent to which the muscle haemoglobin interferes with the estimate by repeating the procedure on leg muscle subsequent to 4 hr. perfusion with Ringer fluid.

Procedure. Four experiments were carried out in each of which twelve sartorii from six killed and pithed frogs were taken. The surfaces of the sartorii were dried between filter paper, then ground to a fine paste with 3 ml. water, the mixture centrifuged and the supernatant fluid collected. The residue of ground muscle was mixed with 3 ml. Ringer fluid, returned to the mortar, ground, centrifuged and the fluid collected. This was repeated until four such extractions were made. The efficiency of the extraction was tested by the examination of further extracts with Ringer fluid or with 10% KOH, using the colorimetric procedure referred to subsequently. It was found that after the fourth extraction less than 5% of the total haemoglobin originally present could have remained, also the residual ground muscle appeared quite colourless. 5 ml. of the collected fluid were taken and 1 ml. of 25% KOH added and mixed.

Blood collected—and oxalated—from 2–6 frogs corresponding with each muscle experiment was diluted 500 times, using the same average diluting fluid as for the muscle extraction, including alkali, to bring to 4.2% KOH. The addition of the alkali to the muscle extract had the advantage of lessening turbidity, which was further diminished by centrifuging at 3500 rev./min. for 30 min. It also served to maintain the haemoglobin in solution as alkali haematin, the change from the reddish yellow of the diluted blood to greenish brown being immediate on adding the strong alkali, no further colour change being apparent on warming.

The extinction coefficients of the extract and diluted blood were then determined from 750 to 434 $m\mu$ and the region of marked change between 494 and 434 $m\mu$ selected for comparison. The limitation of the extinction measurements to this region helped to eliminate the effect of increasing light scatter with diminishing wave-length arising from the slight turbidity of the extract. This effect, which tends to increase the measure-

ment of the circulatory space, can be taken as comparatively small, since the turbidity gave only an apparent extinction of 0.1 at wave-length 729 $m\mu$ (the corresponding diluted blood value being practically zero).

Results. The results are summarized in Table I. The gross values obtained for the vascular space range from 2.1 to 3.2 ml./100 g. muscle with a mean of 2.8 ml./100 g. From this we may subtract 0.5 ml./100 g.

TABLE I

No. of exp.	Total wt. of sartorii g.	No. of blood samples	Total dilution of muscle in extract ml./g.	Differences between extinction coeffs. for $m\mu$ 434 and $m\mu$ 464		Circulatory space in muscle ml./100 g. gross values
				Extract	Diluted blood	
1	0.81	6	18.7	0.25	0.47	2.1
2	0.70	2	21.6	0.27	0.43	2.7
3	0.79	2	19.2	0.29	0.43	2.6
4	1.00	2	15.4	0.39	0.38	3.2
Results on leg muscles after 2 hr. perfusion with Ringer fluid						
5	1.03	—	15.0	0.10	(0.43)	0.7
6	1.04	—	14.8	0.04	(0.43)	0.4

In experiments 1-4, twelve sartorii from six frogs were taken for each experiment. Experiments 5 and 6 give results on single frogs, the bracketed figure for the blood value being the average for previous experiments.

determined on leg muscles from three frogs after perfusion with Ringer fluid for 2-4 hr. and representing the error arising from the muscle haemoglobin as well as that from increasing scatter, due to slight turbidity of the extract, on passing from 464 to 434 $m\mu$. The mean circulatory space of excised sartorii appears therefore as 2.3 ml./100 g. muscle.

DISCUSSION

The interfibre spaces of excised and immersed sartorii as given by inulin and magnesium experiments are 9.6 and 8.2 ml./100 g. respectively and may be taken as 9-10 ml./100 g. when there is no weight change on immersion. The spaces as measured from the diffusion of sodium and chloride into isotonic glucose are 13 and 12.0, and the figure from Donnan calculations applied to chloride is 12.7. These figures indicate a small but real difference between the space occupied by inulin and magnesium on the one hand and chloride on the other. This corresponds to 3 ml. plasma chloride, but may be somewhat less, when we consider that for the four inulin experiments the values ranged from 7.0 to 13.2. The difference is possibly attributable to the circulatory space, assuming that inulin or magnesium does not penetrate appreciably into the capillaries within the

time considered. The experiments of Lavietes, Bourdillon and Klinghoffer (1936) may be cited in support of a delayed equilibrium (over 1 hr.) of such substances as sulphate, and hence magnesium and inulin. As against this there is the fact that the glomerular capillaries and capsule allow even inulin through as fast apparently as water. Though a distinction must be drawn between movement of a solute as part of the whole solution and diffusion across gradients, yet the passage of inulin appears to be of the freest kind through the renal capillaries and the capsular membrane; but the latter retains plasma proteins whereas the capillaries in general do not.

There are also the experiments of McCance [1938], which show that inulin penetrates rapidly into the extra-cellular spaces, no constant difference being determinable between observations made 30 min., and those made 150 min. after injection. Preliminary experiments made here with inulin injections into rabbits show also that inulin equilibria across the general capillary circulation is reached in less than 30 min. Yet it is possible, but unlikely, that such considerations may not apply to the muscle capillaries of the frog, and inulin or magnesium equilibria may be long delayed. It may, however, be safely assumed that these substances will not pass across the membrane of red corpuscles trapped in the circulatory space of the muscle, and this could account for about one-third of the difference between chloride and inulin, leaving the equivalent of about 2 ml. space to be explained. An adsorption explanation of such chloride is apparently outruled by a consideration of the consequent high charge densities on the fibre surface, but what may be considered as a probable cause of the small discrepancy is given subsequent to the following considerations.

While the difference between the interfibre space values determined with inulin diffusion into immersed sartorii and chloride diffusion from fresh excised muscle into isotonic glucose is comparatively small, it is very marked if we use the chloride in the sartorius immersed for some time in Ringer fluid, and consider this as existing external to the fibres. Such chloride (per 100 g. muscle) has been shown to reach 26% of the external concentration within 15 min. [Conway & Kane, 1934], or the original ratio is almost doubled. Here the extra chloride must have passed into the fibres and a quantitative explanation of this movement is given in a subsequent paper.

Considering this chloride entrance, the conditions producing it are probably initiated on the death of the animal or after the excision of the muscle when the circulatory space drops from a probable figure of 7% to one of 2%. During this collapse of the circulation some of the blood

chloride and sodium may enter the fibres and a larger amount be present therein than the expected value from Donnan calculations. This would result in the calculation of the interfibre space being too high. Yet this should not affect the conclusions drawn from Fig. 4, since the chloride free in the interfibre spaces will diffuse out rapidly and the amount is approximately determinable from the diffusion curve. However, if we consider the curves of diffusion in Fig. 4 the dotted straight line projections on to the ordinate are probably not quite correct, and should be somewhat curvilinear, giving slightly lower values for zero time.

Calculations with respect to Donnan equilibria

In such calculations we assumed certain points about the permeability of the muscle membrane which are demonstrated in a subsequent paper. Concerning the free water for solution, it may be said here, that as judged from the equilibrium concentration of urea, this is practically identical with the total water present, or about 80 ml./100 g. [Eggleton, 1930; Conway & Kane, 1934]. A similar result (77 ml./100 g.) was obtained by Hill & Kupalov [1930] from measurements of the vapour pressure. If we interpret the 'osmotically active water' as the free water for solution behind the external membrane of the muscle fibres, then we must take the measure of this as the total free water minus the water in the interspaces. The actual demonstration of the volume of this water by changes in muscle volume on varying the concentration of the external solution depends on the exact permeability of the membrane, and the equilibrium established or approached under the conditions studied. It is only by a realization of the facts presented in a later paper that the exact nature of this problem can be appreciated. It will be shown that the 'osmotically active water' must be considered as not appreciably different from the total water minus the interspace water, and this we may term the 'fibre water'.

Calculation of concentrations in the 'fibre water'

When a substance is present only within the fibres the concentration may be obtained by dividing the amount per kg. muscle by the amount of 'fibre water'. Since the interspace fluid, from the above considerations with respect to inulin and chloride, has a mean value of 0.10–0.13 l./kg. muscle and the total free water is either 0.80 or 0.77, we could consider 0.64–0.70 l./kg. as the free water for solution within the fibres. Taking a mean of 0.67, we may interpret this as corresponding to 0.13 l. interfibre water and 0.80 total free water, or alternatively as 0.10 interfibre water and 0.77 total free water (the latter figure in accordance with

the estimate of Hill & Kupalov, 1930). Although such a mean figure may be in error by 0.03 l./kg. this is scarcely significant for the determination of concentrations in the 'fibre water' with respect at least to investigation of potassium accumulation and the like, as described later. For a substance, therefore, present within the fibres only and not in the external solution we may write

$$C_f = C_m / 0.67,$$

where C_f may be regarded as millimols/l. of 'fibre water' and C_m as millimols/kg. muscle. If the substance is present also in the external fluid this becomes

$$C_f = (C_m - 0.13 C_0) / 0.67,$$

taking 0.13 l./kg. as the interfibre water, C_0 being the millimols/l. of external solution.

SUMMARY

1. The mean inulin 'space' in frogs' sartorii immersed in inulin-Ringer solution was found to be 9.6 ml./100 g. muscle. Individual experiments gave values ranging from 7.5 to 13 ml.

2. The interfibre space for free magnesium diffusion in immersed sartorii was found to be 8.2 ml./100 g. (or calculated as 9.2 ml. when there is no change of muscle volume after immersion).

3. From a study of the diffusion of sodium chloride from muscle in isotonic glucose the sodium chloride outside the fibres represents a mean of 12-13% of the external plasma value (the total chloride being 14%). The figure, however, may be as low as 10% for reasons given in the Discussion.

4. The circulatory space in excised sartorii has a mean value of 2.3 ml./100 g.

5. With the fibre membrane permeable to potassium and chloride (as shown in a later paper) the theoretical chloride within the fibre is only 1.1% of the external value, and the experimental evidence, based either on the balance of the chloride quantities, or on the shape of the chloride diffusion curve into glucose appears to agree with this figure.

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THE CONTROL OF THE EXTERNAL SECRETION OF THE PANCREAS IN CATS

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THE presence of secretory and trophic fibres to the pancreas in the vagus nerves was first demonstrated by Pavlov [1910] on dogs, and this work has since been confirmed by many observers [Anrep, 1916; Babkin, 1924; Tonkikh, 1924; Nakagawa, Kakita & Matsumoto, 1925; Baxter, 1931b; Hebb, 1937; Crittenden & Ivy, 1937] on dogs and rabbits.

With the discovery of secretin more emphasis was laid upon the hormonal control of the pancreas, so much so that Bayliss & Starling [1902], who were unable to repeat Pavlov's observations, suggested that 'the nervous process [of pancreatic secretion] is superfluous and therefore improbable'.

These contradictory views seemed to be reconciled by the work of Mellanby [1925], who showed that secretin was responsible for the water and bicarbonate of the pancreatic juice and that the enzyme content of the juice was determined by the vagus nerves. Secretin, according to Mellanby [1926], is carried into the blood stream in association with the bile salts during their passage through the duodenal and jejunal mucous membranes. The importance of bile in this connexion has been denied by Ivy & Lueth [1927], who found that acid in the duodenum was a more potent stimulus to the pancreas. Finally, doubt has recently been cast by Hammarsten, Ågren & Lagerlöf [1937] upon Mellanby's separation of the hormonal and nervous control of pancreatic secretion. These observers claim to have shown in their work on man that secretin stimulates the production of enzymes by the pancreas.

It was in the hope of obtaining a clearer picture of the control of the external secretion of the pancreas that the following experiments were undertaken.

METHODS

All the experiments were performed on cats. The animals were fed a few hours before the experiment. After preliminary anaesthetization with ether the animals were either decerebrated, or the anaesthesia was maintained by the intravenous injection of chloralose (0.07-0.08 g./kg. body weight). Pancreatic juice was collected by a cannula inserted into the pancreatic duct as it passed through the wall of the duodenum. The ligature round the pancreatic duct included also the bile duct, so that thereafter no bile could enter the duodenum. In a number of experiments the pylorus was occluded by a ligature to prevent the passage of acid from the stomach into the small intestine.

A continuous flow of pancreatic juice at a constant rate was maintained by the intravenous injection of secretin. The secretin was prepared by Mellanby's method [Mellanby, 1932], except that the third stage of purification was omitted. The secretin had no vasodpressor action when tested on the cat's arterial blood pressure. The usual dose was an injection of 0.25 mg. of secretin in 1 c.c. of normal saline every 10 or 15 min., which was sufficient to produce a steady flow of juice at rates between 1.0 to 1.5 c.c. in 10 min. Against this background of continuous secretion it was possible to observe the effect of nerve stimulation and section, and of meals on the rate of flow and the enzyme content of the pancreatic juice.

The amylase and trypsinogen contents of successive samples of about 2 c.c. of juice were measured. The amylase was estimated by Wohlgemuth's method [Cole, 1933]. The diastatic power or diastatic index of the juice ($D_{40/30}$) is expressed as the number of c.c. of 1% starch solution which would be completely converted to erythro-dextrin in 30 min. at 40° C. by 1 c.c. of pancreatic juice. The trypsinogen content of the juice was estimated by allowing 0.25 c.c. of pancreatic juice, activated by the addition of 10 mg. of enterokinase, to digest 5 c.c. of 5% casein solution for 20 min. at 37° C. Formaldehyde was then added and the solution titrated against $N/20$ NaOH with phenolphthalein as indicator. A blank titration was done on a casein sample to which boiled pancreatic juice and boiled enterokinase had been added. To a third casein sample 0.25 c.c. of pancreatic juice and boiled enterokinase were added in order to detect any active trypsin in the juice. The tryptic activity was then converted into amounts of trypsinogen by reference to a graph. The graph was constructed by measuring the tryptic activity of different amounts of a trypsinogen preparation, which was treated in the same way as the pancreatic juice. An arbitrary unit of trypsinogen was taken to be the amount of tryptic activity, measured by the formol titration method, of 0.25 mg. of the trypsinogen preparation, after activation by 10 mg. of enterokinase. The casein solution was prepared as described by Northrop & Kunitz [1932], and the trypsinogen and enterokinase by the methods of Bates & Koch [1935].

The diastatic index and the formol titration give a measure of the concentration of amylase and trypsinogen in the pancreatic juice. More important, however, is the minute output of these enzymes in the various samples of pancreatic juice, and this can be calculated from the diastatic index and the trypsin units by multiplying these by the amount of the sample in c.c. divided by the time of collection of the sample in minutes. In the figures illustrating this paper the terms $D \frac{J}{T}$ and $T \frac{J}{T}$ refer to the minute output of amylase and trypsinogen respectively, calculated in the above manner. The rate of secretion is expressed as the number of c.c. of juice secreted in 10 min.

The experiments fall into two groups, those on the effects of nerve stimulation and nerve section, and those on the effect of foodstuffs.

RESULTS

Stimulation of the vagus nerves

Caudal to the roots of the lungs the right and left vagus nerves form the oesophageal plexus of the vagus round the lower part of the oesophagus. From the oesophageal plexus arise the ventral and the dorsal vagus trunks, which pass through the diaphragm on the ventral and dorsal aspects of the oesophagus respectively, and it is by these two nerve trunks that the vagus nerves reach the abdomen. The ventral vagus trunk is mainly distributed to the stomach, while the major portion of the dorsal vagus trunk passes by its coeliac division to the coeliac ganglia (Fig. 1).

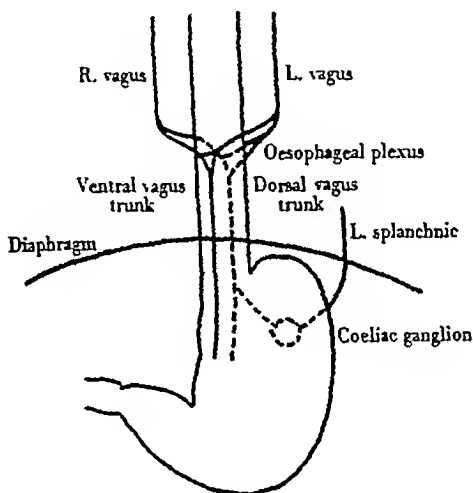


Fig. 1. The abdominal vagus nerves.

One or both of the vagal trunks were sectioned on the lower part of the oesophagus, and the peripheral end of the cut nerve stimulated through bipolar electrodes, by faradic shocks from a du Bois-Reymond induction coil, usually for a period of 5 min.

Stimulation of the dorsal vagus trunk resulted in a well-marked increase in the minute output of enzymes by the pancreas (Fig. 2). The increases in trypsinogen and amylase ran parallel. The increase in enzymes on stimulation of the dorsal vagus trunk was unaffected by occlusion of the pylorus by a ligature, or by previous section of the ventral vagus trunk. After excision of the coeliac ganglia stimulation of the dorsal vagus trunk still brought about an increase in the enzyme content of the

pancreatic juice, from which we conclude that all the 'trophic' fibres to the pancreas do not pass in the major division of the dorsal vagus trunk to the coeliac ganglia. When the splanchnic nerves were cut in the course of experiments, the effect of stimulation of the dorsal vagus trunk on the minute output of enzymes was considerably enhanced compared with the response to stimulation before section of the splanchnic nerves. Large doses of atropine sulphate (1 mg./kg. body weight, intravenously) abolished the response to stimulation of the dorsal vagus trunk.

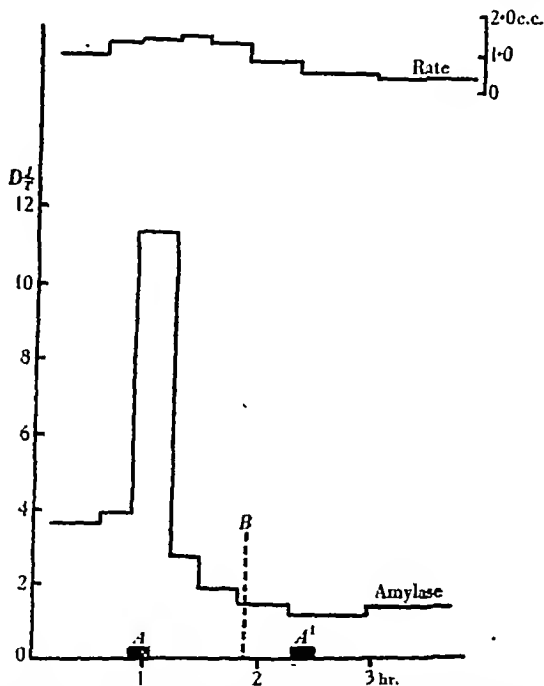


Fig. 2. Stimulation of the peripheral end of the dorsal vagus trunk at *A* resulted in an increase in the minute output of amylase in the pancreatic juice. After the intravenous injection of 5 mg. of atropine sulphate at *B*, repetition of the stimulation at *A'* was without effect.

The effect of dorsal vagus trunk stimulation on the rate of secretion is in sharp contrast to the well-marked increase in the minute output of enzymes. In none of our experiments did we observe any inhibitory effect on the rate of secretion, and in only two out of thirty-one experiments was there any increase in the rate of secretion. In all others the rate was unaltered during stimulation. As an alteration in the position of the cannula occasionally alters the rate of flow of the juice, and as this

cannot always be avoided when stimulating the dorsal vagus trunk in the lower part of the thorax, we feel that such an alteration is the most probable explanation of the two experiments which showed an increase in the rate of flow. In view of the large number of experiments where the rate remained constant while the enzyme output was markedly increased, we conclude that stimulation of the dorsal vagus trunk in the cat does not affect the rate of secretion of pancreatic juice.

Stimulation of the peripheral end of the cut ventral vagus trunk was without effect on either the enzyme output or the rate of secretion by the pancreas.

Section of the vagus nerves

The effect of section of the vagus trunks during the period of collection of pancreatic juice was variable. In some experiments it was followed by a diminution in the minute output of enzymes and rate of secretion. This effect in some cases passed off in $\frac{1}{2}$ -1 hr.; in others it persisted for the rest of the experiment. Again, in other experiments section of the vagal trunks did not affect either the enzyme output or the rate of secretion.

In animals in which the splanchnic nerves as well as the vagal trunks had been sectioned before the cannulation of the pancreatic duct, the minute output of enzymes in the pancreatic juice was high, which showed that the integrity of the vagus supply to the pancreas was not necessary for the secretion of a juice rich in enzymes.

Section of the splanchnic nerves

In animals in which the splanchnic nerves were not cut before the abdominal section for the insertion of the pancreatic cannula there was no secretion of pancreatic juice unless secretin was injected intravenously. In experiments in which the splanchnic nerves were sectioned extra-peritoneally before the abdominal section was made, there was a 'spontaneous' secretion of pancreatic juice, without the injection of secretin. The rate of this 'spontaneous' secretion varied in fourteen experiments between 0.6 and 2.0 c.c. The secretion continued in most cases until the experiment was ended 4 or 5 hr. after cannulation of the pancreatic duct, so that in many experiments on splanchnotomized animals it was unnecessary to inject secretin at all. In experiments in which the splanchnic nerves were sectioned during the collection of pancreatic juice the amount of juice secreted in response to secretin was greater after section of the splanchnic nerves than was produced by the injection of the same amount of secretin before section of the splanchnic nerves (Fig. 3).

The enzyme content of the pancreatic juice in splachnotomized animals was very much higher than in animals with the splanchnic nerves intact. The average minute output of amylase in twenty-one experiments in which the splanchnic nerves were cut was 13.8; in twenty-three experiments in which these nerves were left intact the average minute output was 3.3. The average rate of secretion, 1.2 c.c., was the same in both series of animals.

The 'spontaneous' secretion occurred when the pylorus had been occluded by a ligature. In this case the rate of secretion was slower (0.3 c.c.) and the secretion stopped about 3 hr. after the cannulation of the pancreatic duct. The diastatic index, however, was extremely high, so that the minute output of enzymes during the period of 'spontaneous' secretion was as high as in those experiments where the pylorus was not occluded.

The 'spontaneous' flow of juice with a high enzyme content is obtained in splachnotomized animals in which the dorsal and ventral vagus trunks have been cut, a result which is not produced by vagal section alone.

We regard the absence of 'spontaneous' secretion when the splanchnic nerves are intact, and its presence when the nerves are cut as presumptive evidence of a splanchnic reflex bringing about directly or indirectly an inhibition of pancreatic secretion in the former case. The efferent pathway for such a reflex is obviously the splanchnic nerves. The afferent pathway might be in the vagus nerves, in the splanchnic nerves, or in somatic nerves, the inhibitory stimuli in the last case arising from the skin wounds made in inserting the tracheal and vein cannulae and in opening the abdomen. These possibilities were investigated in the following experiments.

If the splanchnic nerves are left intact but the vagal trunks are cut above the diaphragm before insertion of the pancreatic cannula, there is no 'spontaneous' flow of juice, and the enzyme level is low. If now the splanchnic nerves are cut the enzyme output rises immediately and there is an increase in the amount of juice secreted in response to injections of secretin (Fig. 3).

Anaesthetization with 1 % novocain of the skin of the neck and leg before insertion of the tracheal cannula and vein cannula, of the skin of the thorax before section of the vagal trunks, and of the abdominal wall before opening the abdomen, did not, in animals with intact splanchnics, result in a 'spontaneous' flow of juice or a high enzyme output. But subsequent section of the splanchnic nerves was followed by a marked increase in the output of enzymes and an increase in the amount of juice secreted in response to injections of secretin.

These experiments eliminate vagal afferent and somatic afferent pathways, and we conclude that in animals in which the splanchnic nerves are intact, there is, as the result of trauma to the duodenum during the insertion of the cannula in the pancreatic duct, a reflex inhibition affecting the external secretion of the pancreas. Both the afferent and efferent pathways for this reflex lie in the splanchnic nerves. Whether this is

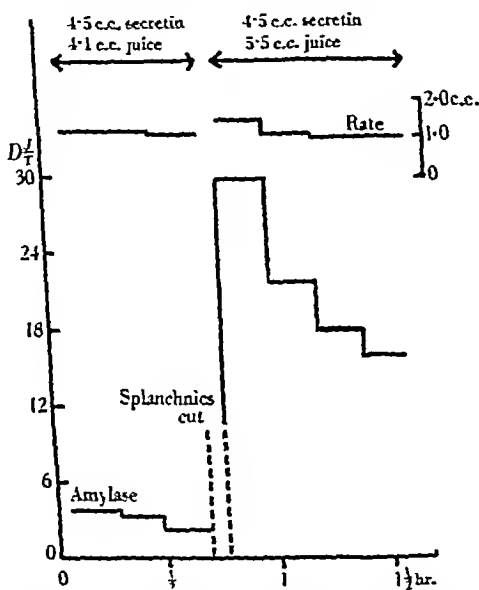


Fig. 3. The vagus nerves were cut before the commencement of collection of pancreatic juice. After section of the splanchnic nerves there was an increase in the amount of juice secreted in response to secretin, and a well-marked increase in the output of amylase.

a direct inhibition of the pancreas through splanchnic fibres supplying the cells or blood vessels of the gland, or whether it is secondary to an inhibition of the absorption of food products from the small intestine will be discussed later.

Stimulation of the splanchnic nerves

When the splanchnic nerves were sectioned in the thorax and the peripheral end of one nerve was stimulated by faradic shocks for a period of 5 min. there was a marked diminution in the minute output of enzymes in the pancreatic juice. During and for some time after the stimulation the rate of secretion was very greatly diminished, a result which may have been secondary to the constrictor effect of the stimulation of the

splanchnic nerve upon the blood vessels of the pancreas. Even, however, when the rate of secretion was kept constant by injections of secretin during and after the stimulation, nevertheless the minute output of enzymes was very much reduced (Fig. 4). From this we conclude that the splanchnic nerves contain fibres which on stimulation diminish the output of enzymes from the pancreas.

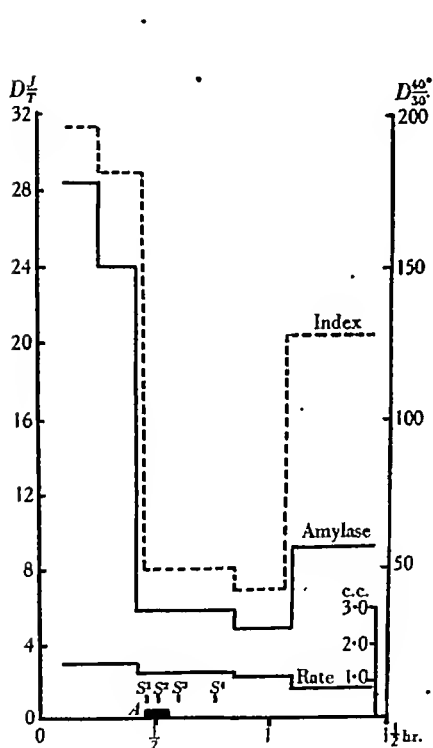


Fig. 4.

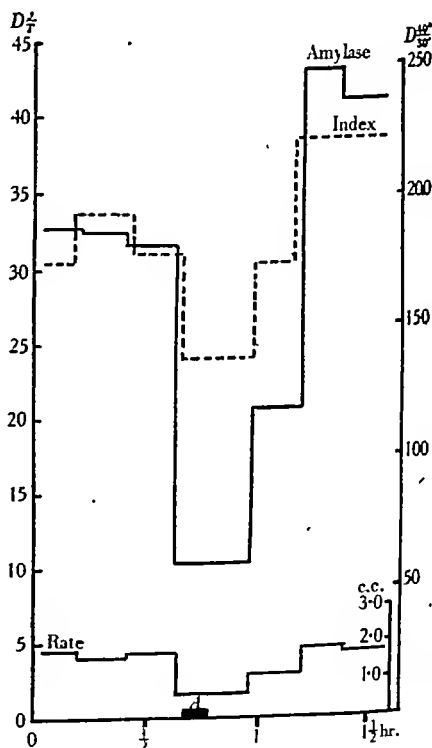


Fig. 5.

Fig. 4. At *A* the peripheral end of the left splanchnic nerve was stimulated in the thorax. At *S*¹, *S*², *S*³ and *S*⁴ injections of 0.25 mg. secretin were given intravenously to keep the rate of secretion of juice constant. For 30 min. following the stimulation the concentration and minute output of amylase in the juice were very much reduced.

Fig. 5. Stimulation of the central end of the left splanchnic nerve in the thorax at *A* was followed by a marked diminution in the rate of secretion, and in the concentration and minute output of amylase.

It has recently been suggested that the splanchnic nerves contain secretory fibres to the pancreas, which, unlike the vasoconstrictor fibres, do not synapse in the coeliac ganglia. In a few experiments we found

that the diminution in enzyme output on stimulation of the splanchnic nerves was much less marked, or even completely abolished after painting the coeliac ganglion on that side with 1% nicotine. In none, however, did we observe any increase in the enzyme output or rate of secretion on stimulating the splanchnic nerve after painting the coeliac ganglion.

Central stimulation of the vagus and splanchnic nerves

The effect of stimulation by faradic shocks of the central end of the cut ventral vagus trunk on the oesophagus was variable. In some experiments there was no effect, in others some diminution in enzyme output and rate. The strength of stimulation had to be kept weak, as strong stimulation produced a violent vomiting reflex. Stimulation of the central end of one splanchnic nerve was in some experiments ineffective; in others it produced a marked diminution in the output of enzymes and the rate of secretion. An example of the latter type of experiment is shown in Fig. 5. In none of our experiments, even with the dorsal vagus trunk intact, did central stimulation of the ventral vagus trunk or of the splanchnic nerves bring about a reflex increase in the enzyme output or rate of secretion.

The effect of the administration of meals

The effects of various foodstuffs on the enzyme output and rate of secretion by the pancreas was studied by the same technique as in the experiments on the innervation of the pancreas. In these experiments the administration of a meal took the place of the nerve stimulation. Normal saline, distilled water, 5% inulin solution, and foodstuffs in the form of 5-8% starch solutions and 5% casein solutions were injected either into the stomach through a catheter passed down the oesophagus, or into the duodenum. The volume of the stomach meals varied between 60 and 100 c.c., the duodenal meals from 30 to 50 c.c. Before the administration of a stomach meal the stomach was emptied and washed out with warm normal saline. Glass cannulae were tied into the duodenum about 4 in. from the pylorus; either a single cannula directed towards the pylorus to collect the stomach outflow, or directed caudally for the injection of meals into the duodenum; or two cannulae were inserted to allow of simultaneous collection of stomach outflow and the administration of foodstuffs into the duodenum. In a number of experiments where meals were injected into the duodenum the pylorus was occluded by a ligature.

Response to stomach meals

The presence of foodstuffs in the stomach did not increase the enzyme output of the pancreas. As soon, however, as the meal began to pass

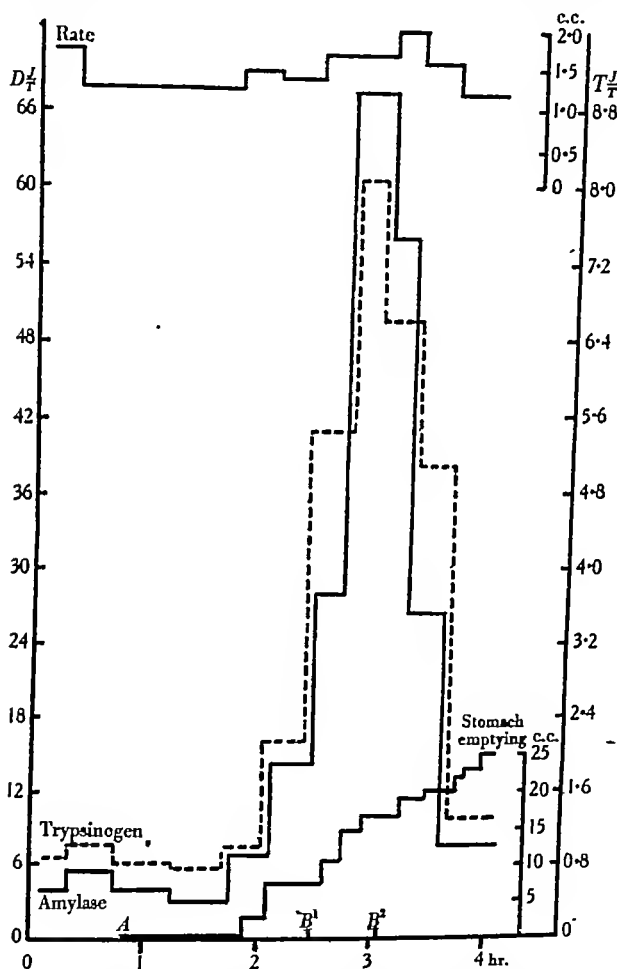


Fig. 6. Besides the usual cannula in the pancreatic duct, two cannulae were inserted into the duodenum, one directed caudally, the other towards the pylorus. At *A* 75 c.c. of an 8% starch solution were run into the stomach. At *B¹* and *B²* 20 c.c. of a partly digested 8% starch solution were run into the duodenum through the lower cannula. For 1 hr. after the administration of the stomach meal at *A* nothing left the stomach and the enzyme output of pancreatic juice remained low. As soon as food passed through the pylorus there was a marked and parallel increase in the output of amylase and trypsinogen, and an increase in the rate of secretion of pancreatic juice. The enzyme output later fell although the stomach was still emptying.

through the pylorus there was a sharp increase in the minute output of enzymes (Fig. 6). The passage of a casein or of a starch meal from the stomach brought about in each case a parallel increase in the amount of trypsinogen and amylase in the pancreatic juice (Fig. 7). During this

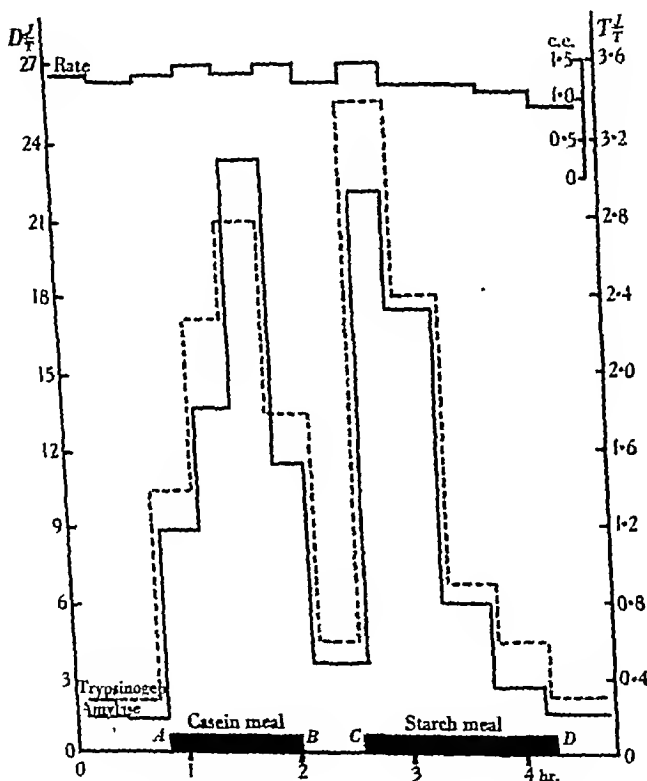


Fig. 7. At *A* 60 c.c. of a 5% casein solution were run into the stomach through a rubber tube passed down the oesophagus. At *B* the stomach was emptied. At *C* a second meal was given, consisting of 60 c.c. of an 8% starch solution. This was removed at *D*. Following the administration of each meal there was a well-marked and parallel increase in the output of trypsinogen and amylase from the pancreas.

period of increased enzyme output there was a measurable amount of active trypsin in the juice. The output of enzymes increased for about 30–40 min. and then declined to resting level over a similar period, even while the stomach was still emptying (Fig. 6). Coincident with the increase in enzyme output by the pancreas there was a definite increase in the rate of secretion of the pancreatic juice, amounting on the average to 50%.

Response to duodenal meals

Results almost the same as those produced by the administration of meals by the stomach were obtained by injecting the meals directly through a cannula into the duodenum. There was a well-marked and parallel increase in the output of trypsinogen and amylase by the pancreas (Fig. 8). In about half the experiments there was an increase in the rate

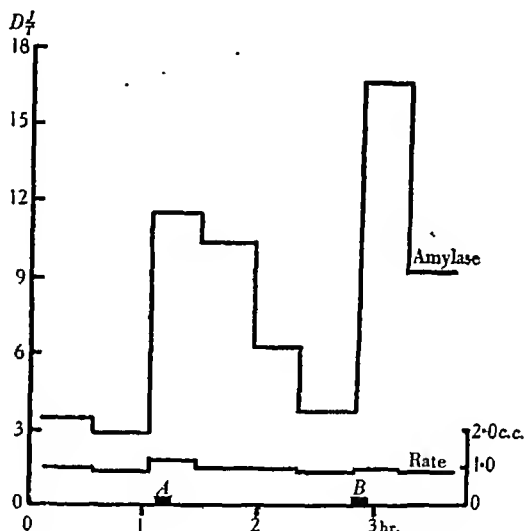


Fig. 8. Effect of duodenal meals. At A 30 c.c. of a 5% inulin solution and at B 30 c.c. of a 5% starch solution were run into the duodenum through a cannula. Following the administration of each meal there was an increase in the enzyme output by the pancreas.

of flow of the juice coincident with the increase in enzyme output. In all those experiments in which the rate of flow increased the pylorus had been occluded by a ligature.

Increases in the enzyme output by the pancreas were observed after the administration by the stomach or duodenum of starch, casein, inulin, saline or distilled water.

The duodenal meals were run into the bowel slowly and under the minimum head of pressure, but in view of the increased output of digestive enzymes on the administration of saline or water or of a non-utilizable substance like inulin, we decided to examine the possibility that distension might stimulate the pancreas. A balloon, 6 in. in length, was passed through the duodenal cannula into the terminal portion of the duodenum and the first part of the jejunum. Distension of the balloon with air to

pressures of 10, 50 and 50 mm. Hg had no effect either on the enzyme output or on the rate of secretion. As a control the balloon was withdrawn and 50 c.c. 5%, starch injected into the same part of the small intestine had the usual stimulating action on the pancreas (Fig. 9). From this we conclude that distension of the bowel is not an adequate stimulus to the pancreas.

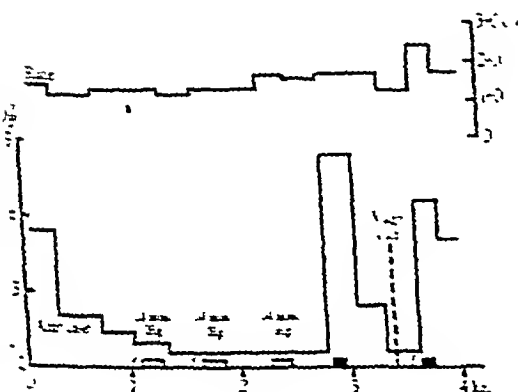


Fig. 9.

Fig. 9. Effect of distension of the duodenum. A balloon 6 cm. in length, was passed into the duodenum. At A, B and C the balloon was distended to pressures of 10, 50 and 50 mm. Hg respectively, without affecting the enzyme output of the pancreas. Control injections of 50 c.c. of a 5% starch solution into the same part of the duodenum at D and E resulted in an increase in the output of amylase both before and after section of the vagus nerves.

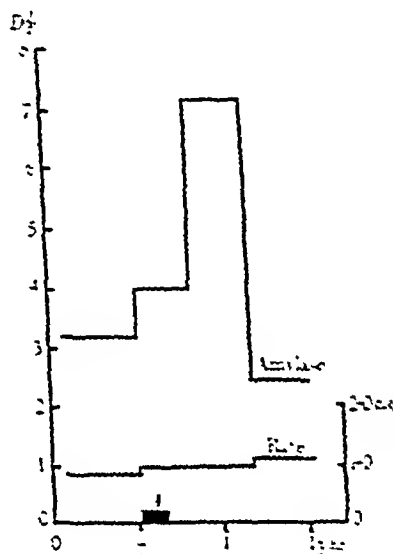


Fig. 10.

Fig. 10. In this animal both vagus nerves, the splanchnic nerves and the inferior mesenteric nerves were cut, and the pylorus and the common bile duct were occluded. At A 50 c.c. of a 5% starch solution were run into the duodenum. Following the administration of the meal there was an increase in the enzyme output by the pancreas.

Effect of nerve sections on the response to meals

The effect of section of the vagal trunks, of the splanchnic nerves, and of the sympathetic fibres from the inferior mesenteric ganglia upon the response of the pancreas to the administration of meals, was studied in a series of animals. The results of these experiments may be summed up in the statement that even when all the extrinsic nerve supply to the small intestine is sectioned (i.e. both vagal trunks cut above the dia-

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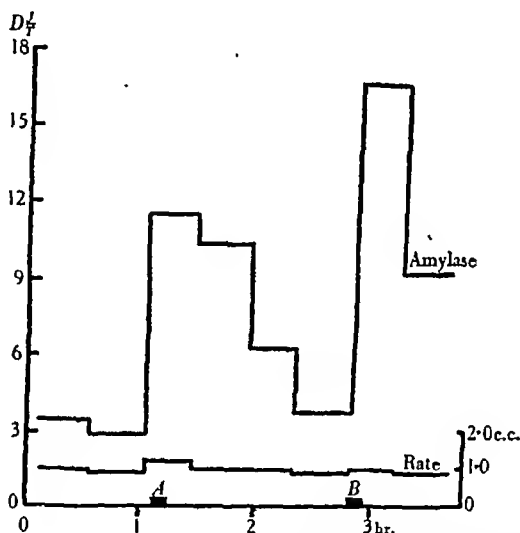


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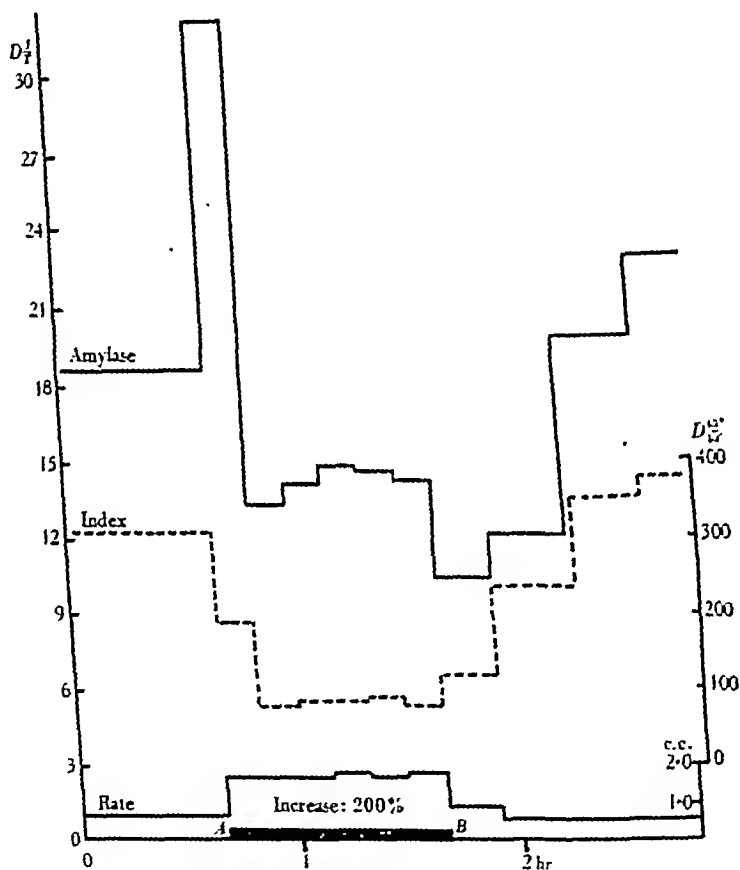


Fig. 11b. Preparation similar to that in Fig. 11a. Injection of secretin at 10 min. intervals between A and B increased the rate of secretion 200% above the 'spontaneous' level. The concentration of enzymes fell during this period, and the minute output of amylase increased during the first 10 min. after A, and thereafter fell below the level of the control samples.

produced a more marked increase in rate (200%), the diastatic index again fell during the period of secretin injections. The minute output of amylase rose sharply in the sample collected during the first 10 min. of the secretin period. Thereafter it fell below the level of the samples collected before the injection of secretin, and did not regain that control level

phragm, the major and minor splanchnic nerves cut extraperitoneally on both sides, and the outflow from the inferior mesenteric ganglia tied off) there is still an increase in the enzyme output by the pancreas on the administration of a meal (Fig. 10).

Effect of secretin upon the output of enzymes by the pancreas

In our search for the mechanism of this stimulation of the enzyme production of the pancreas by meals in an animal with all the extrinsic nervous connexions to the small intestine and pancreas cut, we investigated the possibility that secretin might itself be a stimulant to the

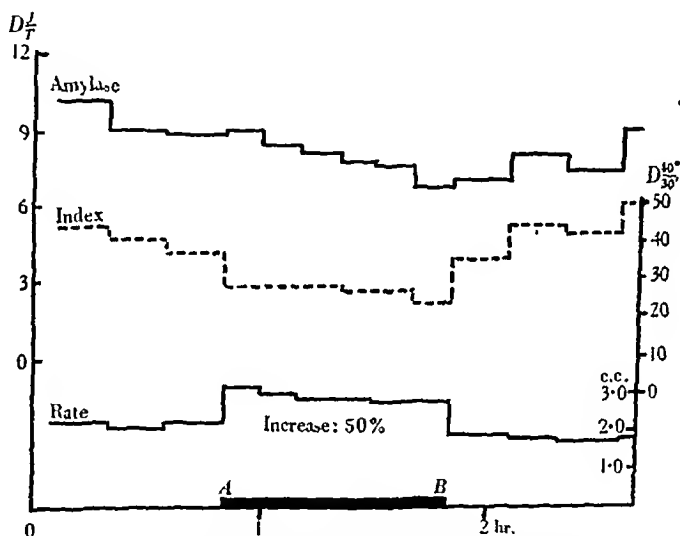


Fig. 11a. Effect of secretin injections in a splanchnotomized animal, which showed a 'spontaneous' secretion of pancreatic juice. Between A and B the 'spontaneous' rate of secretion was increased 50% by intravenous injections of secretin at 10 min. intervals for 1 hr. There was a slight fall in the concentration of enzymes, but almost no alteration in the minute output of amylase.

production of enzymes, as has been suggested by Hammarsten *et al.* These experiments were performed on splanchnotomized animals in which there was a 'spontaneous' flow of juice and a high minute output of enzymes. For a period of 1 hr., at intervals of 10 min., 0.25 mg. of secretin was injected, and a sample of juice collected. The rate and the minute output of amylase were estimated before, during and after the period of secretin stimulation. In an animal in which the spontaneous rate of secretion was high (2 c.c.), and the secretin injection produced only a

moderate increase in rate (50%) there was a proportionate fall in the diastatic index, so that the minute output of amylase remained practically unaltered throughout the experiment (Fig. 11a). Where the 'spontaneous' rate of secretion was lower, and the secretin injections

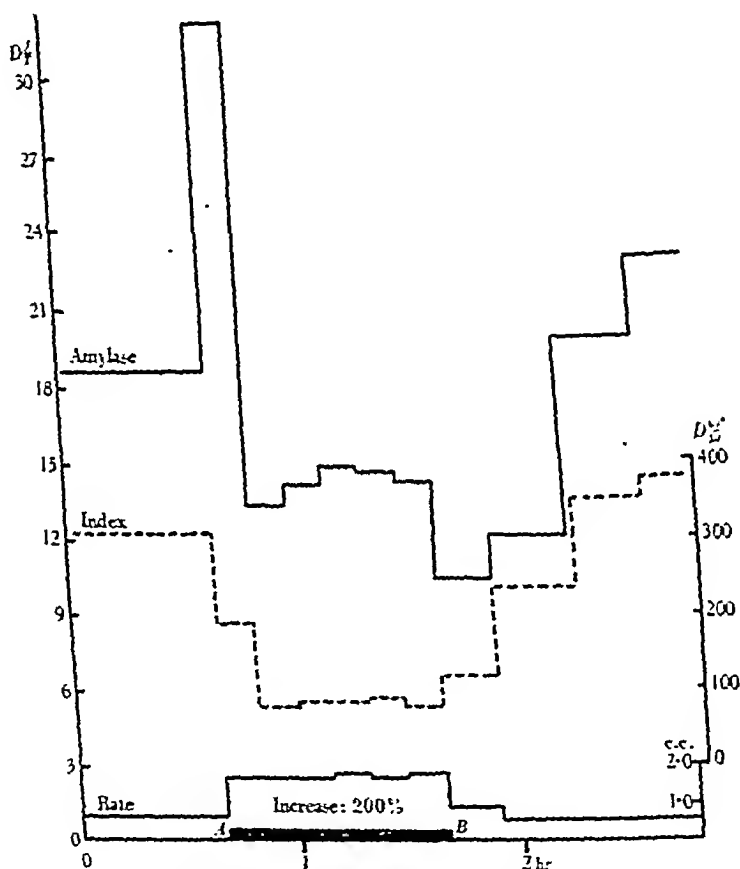


Fig. 11b. Preparation similar to that in Fig. 11a. Injection of secretin at 10 min. intervals between A and B increased the rate of secretion 200% above the 'spontaneous' level. The concentration of enzymes fell during this period, and the minute output of amylase increased during the first 10 min. after A, and thereafter fell below the level of the control samples.

produced a more marked increase in rate (200%), the diastatic index again fell during the period of secretin injections. The minute output of amylase rose sharply in the sample collected during the first 10 min. of the secretin period. Thereafter it fell below the level of the samples collected before the injection of secretin, and did not regain that control level

until some 40 min. after the cessation of the secretin stimulation (Fig. 11b). These experiments show that during the period of increased secretion brought about by the injection of secretin the minute output of amylase is unaffected if the increase in the rate of secretion is moderate. When the increase in rate is marked, the increase in minute output of amylase during the first 10 min. may be due to a washing out of preformed enzymes in the gland cells. If secretin were a true stimulant of pancreatic enzymes one would expect the minute output of amylase to be increased throughout the whole period of secretin stimulation, whereas the minute output was actually diminished for the rest of the hour, and remained below the control level for some time afterwards. We conclude therefore that the secretin preparation which we were using was not a true stimulant to the production of enzymes by the pancreas. In one experiment we replaced an injection of our secretin preparation by one of 'Pancreotest', the secretin preparation used in Hammarsten's experiments. The sample of juice collected after the injection of 'Pancreotest' showed no increase in the diastatic index or in the minute output of amylase.

DISCUSSION

Almost all workers on the external secretion of the pancreas have experimented on dogs or rabbits, and very few observations have been made on the nervous control of the pancreas in the cat. Apart from a short note by Satake [1923] the only work on the effect of vagal stimulation in this animal is that by Korovitsky [1923] and by Sergeyeva [1938]. The probable explanation is that, as noted by these last two observers, vagal stimulation in the cat produces no 'visible' secretion. We have verified this absence of effect of vagal stimulation upon the rate of pancreatic secretion. We have not found any evidence of an apparent inhibition of secretion due to constriction of the ducts of the pancreas on vagal stimulation, as found by Anrep [1916] on dogs, and as one would have expected from the results of Korovitsky on cats.

All the 'trophic' fibres to the cat's pancreas appear to be contained in the dorsal vagus trunk. It was demonstrated by Savitsch [1909] on dogs that the concentration of amylase, lipase and trypsinogen in the pancreatic juice ran parallel, an observation which has been verified by Anrep, Lush & Palmer [1925] on dogs, and by Baxter [1931*a*, 1935] on rabbits. We have found this parallelism in the concentration of trypsinogen and amylase in the pancreatic juice of cats, under different conditions of stimulation of nerves, by secretin, and by protein and carbohydrate meals.

The difficulty of determining whether the splanchnic nerves contain fibres inhibitory to the pancreatic secretion is that the effects observed upon the rate of secretion and the enzyme content of the juice may be secondary to the constrictor effect of the splanchnic stimulation upon the blood vessels of the pancreas. Edmunds [1909, 1911] came to the conclusion that the inhibitor action of adrenaline and of splanchnic stimulation upon pancreatic secretion was not specific, but secondary to the constrictor action. Babkin [1924] concluded that his experiments gave no support to, but did not disprove, the view that there are special inhibitory fibres in the splanchnic nerves to the acinous cells. The diminution in enzyme output in our experiments during and after stimulation of the splanchnic nerves, even when the rate of secretion is kept constant by increased injections of secretin, seems to point to a true inhibitory action of the splanchnic nerves upon the pancreatic cells, although it may be argued that the cutting down of the blood supply to the pancreas would in itself lead to a diminution in the enzymes of the juice. Taking this piece of evidence, however, in conjunction with the 'spontaneous' secretion and high minute output of enzymes in splanchnotomized animals, and with the enhanced response to vagal stimulation after splanchnotomy we feel justified in concluding that the splanchnic nerves contain fibres which are inhibitory to the acinous cells of the pancreas.

Baxter [1931b] showed that in the rabbit the splanchnic nerves contain secretory fibres to the pancreas. Sergeyeva [1938] has brought forward histological evidence, and Babkin, Hebb & Sergeyeva [1939] experimental evidence, that there are secretory and trophic fibres to the pancreas in the splanchnic nerves of the cat, and that these fibres do not synapse in the coeliac ganglia. In some experiments in which we painted the coeliac ganglion on the stimulated side with 1% nicotine, we observed no increase in the rate of the secretion or its enzyme content on subsequent stimulation of the splanchnic nerves. Since we have done only a few experiments of this type, and as in some of these there was still a slight diminution in the rate of secretion on splanchnic stimulation after painting the coeliac ganglion with nicotine (suggesting that the ganglion had not been completely paralysed), and since, moreover, our period of stimulation (5 min.) was very much shorter than the 3-6 hr. of stimulation employed by Sergeyeva, we do not feel justified in drawing any definite conclusion about the existence of secretory fibres in the splanchnic nerves.

the duodenum during cannulation of the pancreatic duct an inhibitory reflex is elicited through the splanchnic nerves. The absence of pancreatic secretion in these cases might be due to a direct inhibitory action of the splanchnic fibres on the secretory cells of the pancreas, to a constrictor action on the blood vessels of the gland, or might be secondary to an inhibition of the absorption from the small intestine of digestion products and secretin which would stimulate the pancreas. In favour of the first possibility is the evidence that the splanchnic nerves contain fibres which have a direct inhibitory effect upon the acinous cells of the pancreas. There seems to be little evidence on the effect of the extrinsic nerves upon absorption from the small intestine. Horne, McDougall & Magee [1934] found that either vagotomy or splanchnotomy increased the absorption of glucose from the small intestine in rabbits. In our experiments vagotomy did not prevent the inhibitory effect on pancreatic secretion. On the other hand the shortening of the period of 'spontaneous' secretion of juice when the passage of foodstuffs and acid from the stomach was prevented by occlusion of the pylorus would suggest that the 'spontaneous' secretion depended upon absorption from the small intestine. The evidence is insufficient to allow us to decide upon the mechanism of the inhibition of pancreatic secretion, but the important practical point is that even slight trauma to the duodenum results in an inhibition of the digestive secretion of the pancreas over a period of at least 4 or 5 hr.

The effect of stimulating the central end of vagal and sympathetic nerves was in most cases to produce an inhibition of pancreatic secretion. In none was there any increase in secretion or enzyme output. Our inability to produce a reflex excitation of the trophic fibres in the dorsal vagus trunk raises the question of how a discharge of impulses along these fibres is brought about in the normal animal. The possibilities would seem to be either a reflex brought about by stimulation of afferent fibres in the vagus or splanchnic nerves from the small intestine, an effect which we have been unable to produce by electrical stimulation of these nerves, or a cephalic reflex either from the taste of food or by psychic stimulation. About this latter mechanism there appears to be some doubt. Crittenden & Ivy [1937] state that meat broth produces a psychic stimulation of the pancreas in dogs, but Villaret & Justin-Besançon [1936], reviewing previous work, conclude that any psychic effect on the pancreas is either feeble or non-existent, and they were unable to demonstrate any psychic secretion in a human subject with a pancreatic fistula.

There are two points of interest in the response of the pancreas to the injection of fluids into the small intestine, the increase in rate of flow and the increase in the enzyme output. Well-marked increases in the rate of secretion were observed on giving duodenal meals after the pylorus had been occluded, so that the release of secretin by the passage of acid into the small intestine was prevented. In all experiments the passage of bile into the small intestine was prevented by the ligation of the common bile duct along with the pancreatic duct, so that this mechanism for the release of secretin from the intestinal mucosa was also excluded. The increases in rate were observed after section of the extrinsic nerves to the gut, so that they could not have been brought about reflexly through the central nervous system. These results indicate that there is some other mechanism for the release of secretin than the association with bile salts [Mellanby], or the action of acid [Bayliss & Starling, Ivy & Lueth].

Even more difficult to explain is the increase in enzyme output from the pancreas following the administration of meals to animals in which all the extrinsic nerve supply to the small intestine has been sectioned. This result, coupled with the very high level of enzyme output in vagotomized and splanchnotomized animals would seem to disprove Mellanby's claim that the vagus is responsible for the enzymes of the pancreas. Nor do our experiments on the effect of secretin injections in splanchnotomized animals lend any support to the suggestion by Hammarsten *et al.* that secretin stimulates the production of enzymes by the pancreas. We are left therefore with the possibilities that the increased enzyme output in response to meals in extrinsically denervated animals may be mediated through the intrinsic nerve plexuses of the intestine (though this is difficult to visualize) or by some humoral mechanism, either secretagogue in type, or some hormone other than secretin. These possibilities are now being investigated.

SUMMARY

1. The effect of nervous and alimentary stimuli on the enzyme output and rate of secretion of the cat's pancreas has been investigated.
2. All the 'trophic' fibres to the pancreas are contained in the dorsal vagus trunk.
3. There is a parallel secretion of trypsinogen and amylase in the pancreatic juice during alimentary, nervous or secretin stimulation of the pancreas.
4. Vagal stimulation has no effect upon the rate of secretion of pancreatic juice.

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THE DISTRIBUTION OF ACETYLCHOLINE IN THE PERIPHERAL AND THE CENTRAL NERVOUS SYSTEM

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LOEWI & HELLAUER [1938] have shown that acetylcholine (ACh.) occurs in high concentration in the ventral spinal roots and in the cervical preganglionic sympathetic trunk, while the dorsal spinal roots, the cervical postganglionic sympathetic trunk, and the optic nerve contain little or none. There is, as they have pointed out, much evidence that the nerves of the former group contain cholinergic fibres, acting through the release of ACh. at their endings. Such evidence is lacking for the nerves of the latter group, an exception, however, being the liberation of ACh. by impulses reaching the periphery in axon branches of some dorsal root fibres [Wybauw, 1936]. Chang, Hsieh, Lee, Li & Lim [1939] confirmed the results of Loewi & Hellauer, and added to the list of nerves rich in ACh. the sciatic, peroneal, hypogastric, phrenic, splanchnic and the brachial plexus. All these contain somatic motor and/or preganglionic autonomic fibres. Lissák [1939] also found ACh. in motor, mixed and preganglionic sympathetic nerves, in contrast to postganglionic sympathetic nerves, which he demonstrated to contain adrenaline. The present paper supplies further evidence that in the peripheral nervous system ACh. is associated with cholinergic fibres, and adds some data on the distribution of ACh. in the central nervous system, where its significance is, as yet, hypothetical.

METHODS

Peripheral nerves and ganglia and brain tissue were obtained from cats anaesthetized with chloralose. Spinal-cord tissue was obtained from dogs anaesthetized with a chloralose-urethane (1:10) mixture. Eserine sulphate (1 mg./kg. intravenously) was given to each anaesthetized

animal, in order to minimize enzymatic destruction of ACh. before the inactivation of cholinesterase by the extracting fluid. Peripheral nerves and ganglia were then dissected clean with as little injury as possible, weighed on a torsion balance, and minced without delay in trichloroacetic acid. Brain tissue was excised with a sharp knife or scissors and similarly treated. Segments of spinal cord were removed and frozen with solid CO_2 , and cylindrical samples taken by passing a biopsy needle of wide bore (1.5 mm.) parallel to the long axis of the cord and expelling the sample from the needle with a trocar. This rather difficult procedure was facilitated by the use of a device, designed by Dr E. J. H. Schuster, by means of which the segment of frozen cord, mounted on a small stage, could be aligned with the sampling needle, which was propelled with a rotatory motion through the tissue by means of a hand-operated screw. Microscopic examination of stained sections of the cord from which the sample had been taken ensured that the needle had followed the intended course.

Extracts of all these tissues were made with trichloroacetic acid as previously described [MacIntosh, 1938], and tested on the eserized dorsal muscle of the leech or on the blood pressure of the cat. The two methods of assay gave identical results: that on the cat's blood pressure [Brown & Feldberg, 1936], however, was the more convenient; and was generally used. Further evidence that the activity of extracts was due entirely to ACh. was supplied by their ineffectiveness after treatment with cold alkali, or when tested on the uneserized leech preparation, or on the blood pressure of the cat after atropine.

RESULTS

Peripheral nervous system. Table I summarizes the results, and shows that nerve trunks containing mainly somatic motor or preganglionic autonomic fibres are regularly rich in ACh. Mixed nerves contain less, and sensory nerves very little. The presence of appreciable quantities of ACh. in the lingual and superior laryngeal nerves is probably to be attributed to parasympathetic components. The small size of some sensory nerves made it impossible to determine their low ACh. content with accuracy. Sympathetic ganglia, which are rich in synaptic junctions, are correspondingly rich in ACh., while the sensory nodose ganglion of the vagus contains about the same proportion of ACh. as the trunk of the nerve below the ganglion. The ACh. present in a unit weight of a particular nerve or ganglion was found to be fairly uniform in the cats examined.

TABLE I. Distribution of ACh. in the peripheral nervous system

Tissue	ACh. content ($\mu\text{g./g.}$)
Nerves containing mainly sensory fibres	
Optic	0.3 (1)
Trigeminal, ophthalmic division	0.8
Trigeminal, maxillary division	0.3, <0.6
Lingual	2.0, <2.5
Auditory	<0.8
Superior laryngeal, internal ramus	1.2, <2.0
Saphenous	0.4, 0.5, <1.2
Dorsal roots, lumbar region	0.04, <0.1
Nerves containing mainly motor fibres	
Oculomotor	15
Facial	6
Spinal accessory	10
Hypoglossal	11, 14, 14
Recurrent laryngeal	13
Phrenic	10, 11, 12, 13, 16
Ventral roots, lumbar region	12, 18
Mixed nerves	
Brachial plexus	6
Sciatic	4, 4, 6
Peroneal	4
Tibial	2.5
Cruial	7
Autonomic nerves and ganglia	
Cervical vagus	6, 9, 9
Preganglionic cervical sympathetic	18, 25, 30
Splanchnic (major)	17
Hypogastric	15
Mesenteric	4
Ciliary ganglion	12
Superior cervical ganglion	18, 36, 37
Stellate ganglion	44
Thoracic sympathetic chain	32
Inferior mesenteric ganglion	40
Nodose ganglion	6, 6, 7

ACh. in degenerating nerves. The phrenic, hypoglossal, or sciatic nerve, the vagus nerve below the nodose ganglion, was cut aseptically on one side in a few cats. The concentration of ACh. in the degenerating nerve, 3 days later, was 3-25% of that in the nerve of the opposite side. Her experiments showed that the disappearance of ACh. is most rapid the 2nd and 3rd days after section. Hellauer & Umrath [1939] and Sák, Dempsey & Rosenbluth [1939] have reported similar results. The disappearance of ACh. from a degenerating nerve is not progressive, distal portions being affected as soon as those near the point of section.

In two experiments, one vagus nerve was cut aseptically above the nodose ganglion. The cervical portions of the nerves, examined 5 and 14 days later, contained 45 and 30% respectively of their original content

of ACh. These results appear to suggest the presence in the nodose ganglion of cells which give rise to efferent fibres, and, in fact, in both experiments, stimulation of the partly degenerated nerve in the neck produced a small rise in blood pressure, with no effect on the heart rate. Heinbecker & O'Leary [1933] have described motor effects in the lungs and duodenum, evoked by stimulation of the vagus in similarly operated cats.

Central nervous system. Table II summarizes the results. No sample from the spinal cord or brain was as rich in ACh. as some peripheral nerve trunks and ganglia. In the spinal cord, ACh. is mainly confined

TABLE II. Distribution of ACh. in the central nervous system

Tissue	ACh. content ($\mu\text{g./g.}$)
Spinal cord	
Whole cord (lumbar region)	1.0, 1.3, 1.6, 2.5
Grey matter	1.5, 3.5, 4.0
Grey matter, dorsal horn	2.5
Grey matter, ventral horn	1.5
White matter, dorsal columns	0.02, 0.05, <0.15, <0.2
White matter, lateral	0.35
White matter, antero-lateral	0.8, 1.0
White matter, anterior	0.3, 0.4, 0.8
Brain	
Pyramids	0.2, <0.4, <0.5
Dorsal columns (nuclei probably included)	0.4, 0.4, 0.6
Medulla, oval and elliptical areas	1.2, 2.7
Medulla, trapezium	1.0
Cerebellum	0.18
Pons, superficial medial	3.2, 5.0
Pons, superficial lateral	1.4
Superior corpora quadrigemina	1.6, 1.7
Mid-brain	1.6
Mid-brain, region of origin of III	4.0
Basal ganglia	7.0
Thalamus	1.8, 4.2
Hypothalamus	✓ 1.6, 2.1
Corpus striatum	✓ 2.7
Occipital lobe, cortex	✓ 2.2
Occipital lobe, white matter	0.4
Temporal lobe, cortex	2.4, 4.0
Temporal lobe, white matter	0.7
Parietal lobe, cortex	1.2, 1.7, 2.2, 3.8, 4.0, 4.0
Parietal lobe, white matter	0.3, 0.6, 0.9, 1.0
Frontal lobe, cortex	4.5
Internal capsule	0.25, <0.6, 1.0, 3.3
Corpus callosum	0.9, 1.1, 2.3, 4.2
Olfactory bulb	1.3

to the grey matter, but some is to be found also in the ventral and ventro-lateral white matter, through which the motor axons pass. The afferent fibres in the dorsal columns, as in their course outside the cord, contain only minute amounts. As the dog's cord was too small to permit

the assay of ACh. in other well-defined ascending or descending tracts, an attempt was made to obtain samples of such tracts from the frozen cord of the horse. The ACh. content of the horse material was, however, always very low, possibly because the animals could not be given eserine beforehand.

The data from brain samples do not, in general, permit the deduction that ACh. occurs, or does not occur, in individual tracts and nuclei. Such information could probably be obtained only by study of a larger brain, since the assay of ACh. in samples weighing less than a few mg. is impossible with available methods. One or two exceptions may be noted: the dorsal columns in the medulla, as in the spinal cord, are very poor in ACh.; and it is especially noteworthy that the pyramids, which are composed almost entirely of the axons of internuncial neurons, likewise contain little or no ACh. ACh. is found in both grey and white matter of the cerebral hemispheres, but in all regions the grey matter contains more than the white. This might suggest concentration of ACh. at synapses, or in relation to cell bodies, and in agreement with this, ACh. occurs in fairly high concentration in the basal ganglia and in the mid-brain. On the other hand, there is a significant proportion of ACh. in parts of the corpus callosum and of the internal capsule, and in the superficial layers of the pons, all of which contain few cell bodies; while the cerebellum, which is rich in cells, contains hardly any ACh.

DISCUSSION

The concentration of ACh. in certain nerves, and in various parts of the central nervous system, has been determined by several authors in addition to those already mentioned. Among these may be named Witanowski [1925], Chang & Gaddum [1933], Kwiatkowski [1934], Plattner [1934], Dikshit [1934, 1938], and Barsoum [1935]. In many instances the values obtained by these authors are appreciably lower than those now reported. The discrepancy is due, doubtless, in some cases to the fact that species other than the cat were examined, and in other cases to the fact that eserine was not given before the tissues were removed for extraction, or to the use of less efficient extraction methods.

The present observations support the conclusion of Loewi & Hellauer that, in the peripheral nervous system, cholinergic nerve fibres contain ACh. throughout their course, and other nerve fibres contain practically none. Those parts of the central nervous system which contain efferent

fibres likewise contain ACh. and those parts which contain only afferent fibres contain little or none. In other parts of the central nervous system, in which the arrangement of fibres and cells is more complicated, ACh. is found in very variable concentration. While it is, as yet, impossible to define precisely the structures in which it occurs, it is clear that its concentration bears no definite relation to the relative abundance of either cells or synapses. Certainly it must not be concluded that the presence of ACh. in many parts of the central nervous system signifies the presence there of cholinergic neurons. There are certain tissues, notably the ungulate spleen and the human placenta, which contain much ACh. but no known cholinergic fibres; and it is possible that some of the ACh. of the brain, too, may be located in non-neural tissue, or in neurons which do not require it for the transmission of impulses at their synaptic endings. Better founded, perhaps, would be a deduction that the *absence* of ACh. implies the absence of cholinergic neurons. If this be so, we can conclude that some central nerve fibres, e.g. those of the pyramidal tracts, are not cholinergic. But this deduction also lacks proof, and even in the case of the peripheral nervous system affords no explanation for the apparently well-established liberation of ACh. by antidromic impulses, reaching the vasodilator endings of peripheral axon branches of sensory nerve fibres.

The concentration of ACh. in preganglionic sympathetic nerve trunks sometimes approaches that in sympathetic ganglia. This does not mean that ACh. is not concentrated at ganglionic synapses. Preganglionic fibres form only a small part of the bulk of a sympathetic ganglion, and, unless their intraganglionic portions are much richer in ACh. than their extraganglionic portions, cannot account for the whole of the ACh. extractable from the ganglion. Since, however, the ganglion loses nearly all its ACh. when the preganglionic fibres degenerate, the evidence {Brown & Feldberg, 1936} remains strong that ACh. is concentrated at the synaptic endings of these fibres.

SUMMARY

1. In the peripheral nervous system of the cat, the distribution of acetylcholine is the same as that of cholinergic fibres.
2. In the spinal cord of the dog, acetylcholine occurs in the grey matter, and in those parts of the white matter containing efferent axons, but not in those parts of the white matter containing only afferent axons.

3. In the brain of the cat, the distribution of acetylcholine does not run parallel to that of cell bodies or synapses. The cerebral cortex, and some nuclei and tracts, are relatively rich in acetylcholine; other nuclei and tracts, relatively poor.

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THE EFFECT OF PREGNANCY ON TISSUE LIPIDES IN THE EWE

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DURING the last few years there has been a considerable amount of research upon various dietetic and hormonal factors which influence the lipide content of tissues, especially the liver. Relatively little attention has been paid, however, to the effect of pregnancy upon tissue lipides. Miotti [1900] recorded an abnormal amount of fat in the livers of pregnant rabbits, and this was confirmed by Coope & Mottram [1914], who found a decided increase in the liver fat of cats and rabbits during pregnancy. On the other hand MacLean, quoted by Best & Ridout [1933], failed to demonstrate a constant increase in the liver fat of pregnant rabbits even when these animals received a diet rich in fat; and Snook [1939] found no evidence of fatty infiltration of the liver near term in pregnant rats, rabbits, guinea-pigs and sheep.

Accumulation of fat in the liver is the most striking post-mortem feature in ewes affected with pregnancy toxæmia, and this is often used to confirm the diagnosis made on the history and clinical symptoms. It has been suggested, however, by various authors, e.g. Greig [1929], Van Rensburg [1931], Dayus & Weighton [1931], that fatty infiltration of the liver might be characteristic of the pregnant ewe near term. Such suggestions were presumably based on post-mortem experience, as no data are quoted in support of the contention.

While there are numerous studies of liver lipides in small experimental animals, little analytical work has been done on the liver lipides of sheep. Turner [1930] analysed five specimens of liver from non-pregnant sheep, whilst Hilditch & Shorland [1937] made a more comprehensive analysis of the livers of twenty-two sheep, fractionating the fatty acids obtained. Roderick, Harshfield & Merchant [1933] reported

that in six normal pregnant ewes the fat content of the liver amounted on an average to 7% of the dry tissue (about 2% of the fresh liver) as compared with 60% of the dry tissue in eight cases of pregnancy toxæmia. Snook [1939] found that in three liver samples from non-pregnant sheep the average chloroform extract amounted to about 5% of the wet liver as compared with an average of 5.5% for specimens from twelve healthy pregnant ewes.

In view of the diagnostic importance often attached to the post-mortem finding of fatty infiltration in the liver of ewes suspected of pregnancy toxæmia, it seemed desirable to make a comprehensive survey of the lipide content of the liver in healthy pregnant and non-pregnant ewes. This was rendered possible by the co-operation of Sir Joseph Barcroft, who was making a study of the development of the foetal nervous system in sheep, necessitating the killing of in-lamb ewes at various stages of pregnancy. Arrangements were made to obtain specimens of liver from these animals and in addition a number of random specimens from pregnant and non-pregnant ewes were obtained from other sources. Gilruth [1924] and we ourselves have observed that in pregnancy toxæmia the kidneys also are frequently pale and fatty. Occasional specimens of kidney from healthy pregnant and non-pregnant ewes were also analysed to see if the lipide content differed in the two groups.

METHODS

For determination of the moisture content 0.5–1.0 g. of tissue was taken and occasionally specimens were preserved for histological examination. The total lipides were extracted by the method of Best, Channon & Ridout [1934] from about 20 g. of tissue which were accurately weighed and then ground up with anhydrous sodium sulphate. A portion of the extract was evaporated down and the lipid residue dried and weighed. Another portion was saponified and the unsaponifiable fraction and total fatty acids estimated by Lieberman's method [Leathes & Raper, 1925]. The mean molecular weight of the fatty acids was obtained by titration [Leathes & Raper, 1925] and the iodine value by Wijs's method. Free cholesterol and total cholesterol were estimated gravimetrically using digitonin precipitation of the original material and of the unsaponifiable fraction respectively. Lipoid phosphorus was determined on an aliquot portion of the original extract by an adaptation of the method of Stewart & Hendry [1935]. In occasional specimens the phospholipins were precipitated with acetone and $MgCl_2$ and weighed separately as a check on the lipid P estimation.

From the data so obtained the following were calculated:

- (a) Combined cholesterol (=total-free).
- (b) Fatty acids combined with cholesterol (as oleic acid).
- (c) Phospholipins (as di-oleo-lecithin).
- (d) Phospholipoid fatty acids (as oleic acid).
- (e) Neutral-fat fatty acids (as oleic acid)=total fatty acids-(fatty acids combined with cholesterol+phospholipoid fatty acids).
- (f) Neutral fat (as triolein) calculated from (e).

RESULTS AND THEIR INTERPRETATION

A summary of the results obtained is given in Table I, which shows the average amounts of the various constituents along with the standard error of the mean. The number of observations on which the result for a particular constituent is based is given in brackets after the standard error in those cases in which not all the specimens in a group were examined for that constituent.

The specimens from non-pregnant ewes (group I) were random samples obtained over two successive years at intervals during the period when pregnancy normally occurs, viz. October to April, and animals of all ages have been included; some specimens came from the same flocks as the pregnant animals studied. The results obtained are in fairly close agreement with those of previous workers. Thus Turner [1930] found that the total *fatty acids* averaged 5% of the moist tissue and had an iodine value of 120 and a mean mol. wt. of about 300. Hilditch & Shorland [1937] showed that on an average the liver contained 5.4% of ether-soluble material, about half of which was phosphatide and the remainder glyceride plus unsaponifiable material; whilst Snook [1939] found that in three samples from non-pregnant sheep the average chloroform extract amounted to about 5%. The average phospholipin content and average total lipides shown in Table I are somewhat greater than those found by other workers, but the difference is possibly due to the more thorough *methods of extraction* adopted.

Group II. The ninety specimens obtained from apparently healthy pregnant ewes were also spread over two successive breeding seasons. It will be seen that on an average there is a 60% increase in the lipides when compared with the non-pregnant animal, due practically entirely to an increase in the neutral fat fraction. A slight but statistically significant increase in the unsaponifiable material other than cholesterol was also noted, but since the exact nature of the fraction is unknown the significance of the finding is obscure. The moisture content of the liver

and also the iodine value of the total fatty acids present were both somewhat lower in group II than in group I, but the figures given do not reflect the real magnitude of this change as they represent the means only about one-third of the specimens. When the moisture content of individual specimens was plotted against the total lipid content a graph obtained showed a slight but definite fall which was linear in nature. This was confirmed by the fact that the coefficient of correlation worked out at -0.82 , showing a high degree of relationship between the moisture content and the percentage of liver fat. The iodine value also falls steadily as the liver fat increases, the coefficient of correlation being -0.72 . These figures suggest a transference to the liver of the monosaturated glycerides from the fat depots, causing in consequence both an increase in liver weight without any increase in moisture content and a fall in the average iodine value of the fatty acids present.

Fig. 1 shows the lipid content of the livers of individual sheep of group II plotted against duration of pregnancy. During the first 2 months the majority of the points fall within the range for non-pregnant ewes but after that there is a definite gradual rise in the lipid content of the liver as pregnancy progresses. In spite of considerable individual variation there is obviously a relationship between the duration of pregnancy and the total lipid content of the liver. This is confirmed by the χ^2 test, which gives a probability value of much less than 0.01 as compared with the conventionally significant value of 0.05 .

The marked individual variation is well shown in Fig. 1, values as high as 19.5% total lipides (practically 60% of the dry matter of the liver) being obtained from apparently normal healthy pregnant ewes. On the other hand at all periods of pregnancy a few specimens were within the range found for non-pregnant animals. Fig. 2 shows the same results taken in monthly groups and the average total lipides plotted against duration of pregnancy. This shows clearly the gradual increase in the lipid content asserting itself during the second month of pregnancy and rising to a maximum of about 12% in the fourth month, after which the average percentage of lipides remains fairly steady until parturition occurs.

Many of the ewes carrying two or more lambs had livers containing large quantities of fat, and it was thought possible that mainly these specimens might be responsible for raising the average fat content of the liver. A statistical analysis of the results for sixty-three ewes with only one foetus each, and twenty-seven ewes carrying two or more foetuses, showed that with every constituent the difference between the means

(groups IIa and IIb, Table I) was much less than the standard error of the difference between the means, so that none of the differences observed can be regarded as being of significance. This is shown graphically in

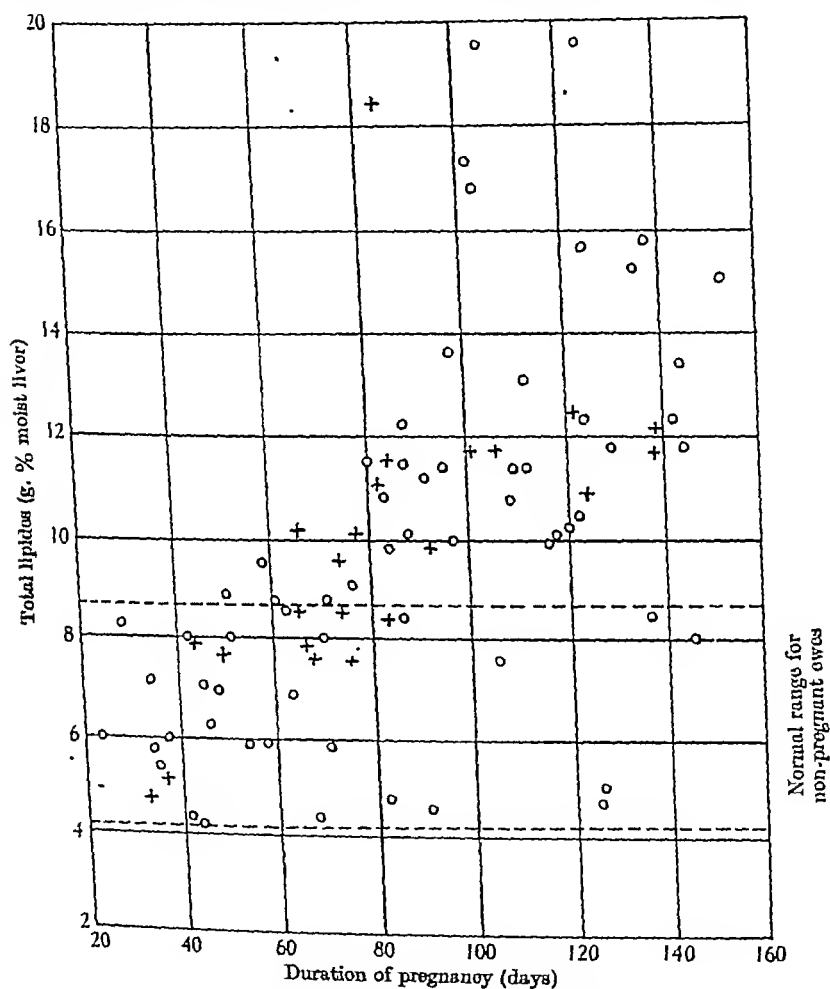


Fig. 1. o Single pregnancies. + Multiple pregnancies.

Fig. 2, where the monthly averages for single pregnancies and for multiple pregnancies are also recorded, and it would appear, therefore, that multiple pregnancy has much the same effect, both qualitatively and quantitatively, as that of single pregnancies.

The increase in fat with the progress of pregnancy might be ascribed to the growing demands of the foetus causing increased fat transport

TABLE I

Lipides present in moist tissue, g./100 g.

Group	Number of specimens	Moisture content %	Other un-saponifiable material					Total lipides	Mean mol. wt. of total fatty acids	Iodine value of total fatty acids
			Phospho-lipides	Free cholesterol	Cholesterol esters	Neutral fat				
I. Livers from non-pregnant ewes	25	70 ±4.7 (15)	3.56 ±0.10	0.20 ±0.017	0.17 ±0.01	1.86 ±0.08	0.04 ±0.16	300	±18 (15)	±7.3 (15)
II. Livers from pregnant ewes	90	66.8 ±2.5 (32)	3.36 ±0.03	0.23 ±0.008 (50)	0.14 ±0.008 (50)	0.59 ±0.01 (50)	0.74 ±0.38	292	±12 (25)	±4.5 (25)
IIa. Livers from ewes with single foetus	63	66.0 ±1.8 (23)	3.36 ±0.03	0.24 ±0.008 (39)	0.145 ±0.000 (39)	0.54 ±0.01 (39)	0.73 ±0.50	290	±19 (16)	±7.0 (16)
IIb. Livers from ewes with more than one foetus	27	67 ±4.6 (9)	3.35 ±0.15	0.21 ±0.02 (11)	0.14 ±0.02 (11)	0.74 ±1.15 (11)	0.75 ±0.07	295	±35 (9)	±11.0 (9)
III. Livers from castrated male sheep	6	68 ±20.0 (4)	3.45 ±0.60	0.27 ±0.05	0.17 ±0.04	7.55 ±0.25	11.60 ±2.00	297	±125 (3)	±22.0 (4)
IV. Livers from suspected cases of pregnancy toxemia	6	60 ±4.0	3.49 ±0.65	0.23 ±0.05	0.14 ±0.03	8.45 ±2.40	12.04 ±3.00	290	±69 (4)	±22.0 (4)
V. Kidneys from non-pregnant sheep	10	80 ±8.6	2.41 ±0.30	0.33 ±0.04	0.12 ±0.02	0.41 ±0.14	3.20 ±0.04	323	±72 (5)	±40.0 (5)
VI. Kidneys from pregnant ewes	35	70 ±4.3 (26)	2.23 ±0.69	0.32 ±0.008	0.125 ±0.01	0.61 ±0.01	3.36 ±0.13	323	±17 (20)	±7.0 (20)
VII. Kidneys from cases of suspected pregnancy toxemia	2	80.4 ±0.4	2.30 ±0.40	0.31 ±0.01	0.13 ±0.07	0.52 ±0.49	3.32 ±0.00	321	±13	±2.0

from the body depots to the liver and the accumulation of fat in that organ. But the growth curve of the foetus as obtained from some forty determinations of foetal weight (Fig. 2) shows that the maximum increase occurs in the last month of pregnancy, by which time the liver fat has already reached its maximum. During the 3rd and 4th months of pregnancy, when the liver fat is increasing to its maximum level, the foetus gains about 1 kg. In the last month of pregnancy, however, the

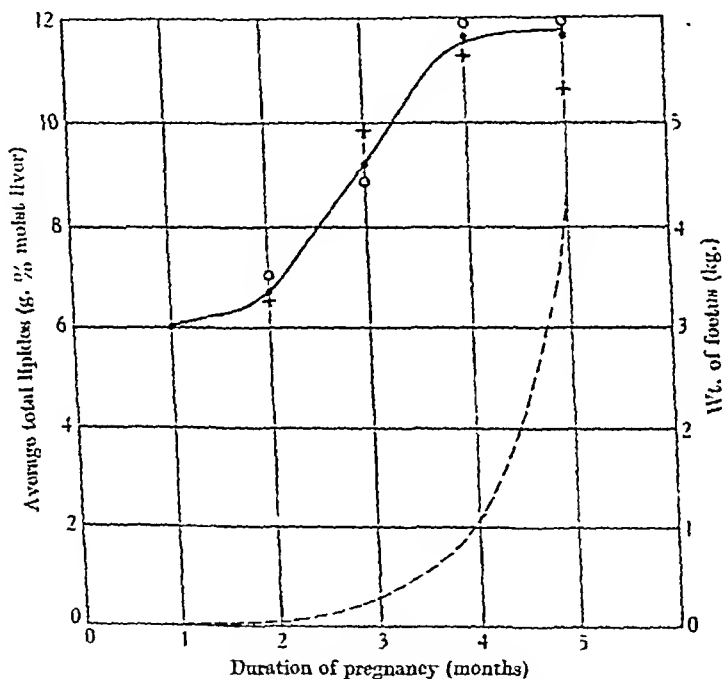


Fig. 2. — Average liver lipides. --- Growth curve of foetus.
 o Single pregnancies. + Multiple pregnancies. • All cases.

weight of the foetus increases rapidly by about 3 kg., whilst the average liver fat content remains steady. Moreover, in the case of multiple pregnancies the demands on the maternal organism are presumably greater than in single pregnancies, yet there is no significant difference in the fatty infiltration between the two groups. It thus seems unlikely that the infiltration observed can be a quantitative expression of the foetal demands. A more likely explanation of the observed facts would be that some factor was causing mobilization of fat in the liver at a greater rate than required until the last month of pregnancy, when the greatly increased foetal demands stimulated utilization of fat by the

liver at about the same rate as it was being brought in, so that the fat content then remained comparatively constant.

In a few cases liver specimens were obtained from healthy widders (castrated male sheep) slaughtered for human consumption. The liver fat content of these animals (Table I, group III) was much higher than in non-pregnant ewes, being of about the same order as the figures for pregnant animals. Here again considerable individual variation occurred, but the neutral fat fraction was noticeably and significantly increased.

Group IV. Only six cases of pregnancy toxæmia became available during the period of this investigation and in some of these cases the diagnosis was doubtful. The average total lipide content of the liver (12.6%) was slightly higher than the average for normal healthy pregnant ewes in the last month of pregnancy (11.8%). However, as the diagnosis was by no means certain in every case, and as the observers Snook [1939], Roderick *et al.* [1933] quote the liver lipide content in cases of pregnancy toxæmia as being usually about 15–20%, the significance of the figures obtained by us remains rather doubtful.

The results obtained from analyses of kidney lipides are shown in groups V, VI and VII. There are no previous figures on record for kidney lipides in the ewe, but the results obtained are in close agreement with those found for other species. The figures for pregnant animals show a slight increase in neutral fat content, mainly due to a few specimens obtained from ewes approaching parturition which showed a slight tendency towards fatty infiltration, but the difference is barely large enough to be significant. In the two cases of suspected pregnancy toxæmia examined the analyses came within the normal range.

Histological observations

Small portions of tissue were examined histologically using Scharlach red or Sudan III to stain the fat globules. The picture obtained conformed to the biochemical findings. The specimens of liver from pregnant ewes showed usually a considerable degree of true fatty infiltration, commencing in the cells at the periphery of the lobule and spreading gradually inwards until in severe cases the bulk of the lobule was infiltrated. The fat appeared as large globules distending the cells and pushing the nuclei aside. No signs of cell degeneration were seen. In a few kidney sections which showed fatty changes, i.e. those from ewes near the end of pregnancy, the fat globules appeared mainly in the cells of the proximal convoluted tubules and the limbs of Henle.

DISCUSSION

These results bear out the view held by many veterinary surgeons, who, on the basis of post-mortem experience, have claimed that fatty infiltration of the liver is a common finding in healthy pregnant ewes. They contrast with the results obtained by Snook [1939], who found no evidence of fatty infiltration in the livers of twelve healthy pregnant ewes but found considerable fatty infiltration in the livers of seven ewes rendered ketonaemic by dietetic deficiency.

It has been common in the past to attribute the accumulation of fat in the liver to the action of toxic agents on the liver cells preventing the liver from disposing of the fat which is being transported there. It is now known, however, that with diets rich in fat, a deficiency in the intake of choline or its analogues, or of certain proteins, may lead to the production of fatty liver. It seems unlikely that any of these dietetic factors could be the cause of the fatty infiltration we have observed, since the diet of the sheep contained less than 2% of ether-soluble material and there was an adequate protein intake. Moreover, the majority of the non-pregnant ewes from which we obtained the samples were on the same diet as the pregnant ewes or on a closely similar diet.

The general conclusion of Snook [1939] and of Fraser, Godden, Snook & Thomson [1938, 1939] was that ketosis and fatty infiltration of the liver in pregnant ewes are associated with continued under-nutrition or temporary lack of food, that they are inversely correlated with the calorific value of the diet, and that multiple pregnancy accentuates this susceptibility of the ewe to dietetic deficiency. Thus Snook [1939], who obtained a few liver samples from the Cambridge sheep and noted quite a marked fatty infiltration on histological examination, suggested that the cause was unsuspected under-nutrition associated with 'variable food consumption' by the ewes. A scrutiny of the diet of the animals concerned shows that this is extremely unlikely. They were grazed on good pasture all through the winter and in addition each received a daily ration consisting of 1 lb. crushed oats, $\frac{3}{4}$ lb. bran and 4 lb. mixed hay and chaff. The starch and protein equivalents of this extra ration amount to 1.8 and 0.25 lb. respectively, which is ample for the requirements of a ewe even in the absence of grazing, and is more liberal than the diet supplied by Fraser *et al.* [1938] to those ewes fed to over-fatness. Moreover, all the Cambridge sheep thrived well and put on weight normally throughout the duration of pregnancy, and as all the sheep were being fed up to the time of slaughter there is no question of fasting causing a

sudden mobilization of depot fat. Thus though we have no check on the amount of food consumed by individual ewes it is reasonably certain that the flock as a whole received ample supplies of food, so that the steady rise in liver fat which is expressed as an average of the large number of specimens examined is not likely to be due to malnutrition. It is also clearly established in this investigation that multiple pregnancies did not accentuate the condition, and thus we cannot lend support to the hypotheses of Snook [1939] and Fraser *et al.* [1939] previously mentioned.

It is of interest to note the findings by Fraser *et al.* [1939] that in barren ewes neither long-continued maintenance on an inadequate diet nor this treatment followed by a 2-day fast at the end of the period will produce ketonaemia, but that pregnant ewes are much more susceptible to ketosis under such treatment, especially in the latter third of gestation. Now this is the period when according to our findings the liver will have already undergone considerable fatty infiltration, and it was probably the disturbance in the carbohydrate metabolism at this juncture by fasting which accounted for the ease with which such animals were rendered ketonaemic as compared with non-pregnant ewes in which the liver fat is within normal limits.

We have shown that the excessive fatty infiltration in the sheep examined by us is coincident with pregnancy, is independent of the number of foetuses and does not seem to be due to a dietetic factor. It has, however, been established by various workers [Riddle & Flemion, 1928; Best & Campbell, 1936, 1938 and Mackay & Barnes, 1937] that extracts of anterior pituitary can produce an increase in liver lipides. Further, Anselmino & Hoffman [1936] have shown that such preparations made from the pituitary of sheep are particularly potent in producing fatty infiltration of the liver in experimental animals. It is possible that pregnancy may stimulate the over-production of this fat metabolism hormone, with the results herein described. This possibility has not been eliminated in the present study and is being further investigated.

SUMMARY

1. Analysis of specimens from twenty-five non-pregnant and ninety pregnant ewes showed that fatty infiltration of the liver was quite common in apparently healthy pregnant ewes, the average lipide content being about 10% (range 4.3–19.5%) of the moist liver as compared with 6% (range 4.3–8.7%) for the non-pregnant animals.

2. During the first 2 months of pregnancy the amount of lipide present was usually within the normal range for non-pregnant ewes.

Thereafter it rose rapidly and by about the end of the 4th month had reached its maximum value, averaging about 12% total lipides. The average liver-fat level then remained comparatively constant until the end of pregnancy.

3. The increase in lipide in the pregnant group was mainly in the neutral fat fraction, though there was a small increase in the unsaponifiable material. There was a marked fall in the iodine value of the fatty acids present and a decrease in the moisture content of the liver.

4. Multiple pregnancy had no effect on this infiltration and there appeared to be no correlation between the lipide content of the liver and the growth of the foetus.

5. Liver specimens from a few castrated male sheep had a lipide content very similar to that obtaining in pregnant ewes near term.

6. Specimens from a few cases of suspected pregnancy toxæmia in ewes showed a liver lipide content only slightly greater than the average for apparently healthy pregnant ewes at a similar stage of pregnancy.

7. The lipide content of the kidneys showed no significant difference between pregnant and non-pregnant animals.

The significance of these findings is discussed.

We wish to express our thanks to Prof. Sir Joseph Barcroft for his assistance in supplying us with much of the animal tissues required in this investigation. The expenses of this research were defrayed by Grants from the Agricultural Research Council, to whom our thanks are also due.

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THE IDENTITY AND ORIGIN OF THE ANTI-COAGULANT OF ANAPHYLACTIC SHOCK IN THE DOG

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IN 1909 Biedl & Kraus, and Arthus independently, reported a marked increase in the clotting time of the blood of dogs in anaphylactic shock. Earlier, in 1880, Schmidt-Mülheim had noted the increase in the clotting time of the blood which followed an intravenous injection of Witte peptone into dogs. This observation was quickly confirmed, and already by the time of Morawitz's review on the subject of blood clotting in 1905, a considerable literature had accumulated on the nature of the blood changes induced by peptone. It is not surprising therefore that the prolonged coagulation time noted in anaphylaxis in the dog should be compared with, and considered to be fundamentally the same as, that accompanying peptone shock. This comparison appears to have been first made by de Waeles, but he was severely criticized and his views did not meet with general acceptance. Fano had suggested that peptone caused the production within the organism of a substance which was responsible for this prolongation of the clotting time, and that this substance was active both *in vivo* and *in vitro*. The work of a number of investigators, including Delezenne, Contijean, Doyon, Popielski and Nolf, pointed to the liver as a source of this substance (the above mentioned references will be found in the reviews of Morawitz [1905] and Wöhlisch [1929]). While some investigators considered the anticoagulant to arise from a number of different tissues, Nolf, in particular, stressed the view that it was solely of hepatic origin. Certain of these earlier workers had attempted to identify this anticoagulant, but it remained for Howell [1925] to suggest that it might be heparin, the anticoagulant he had obtained from dog liver. Recent investigations have further supported Howell's contention that heparin is responsible for the incoagulability of the blood in peptone shock [Quick, 1936; Wilander, 1939] and also in anaphylactic shock [Eagle, Johnston & Ravdin, 1937].

We have isolated heparin in crystalline condition from the blood of dogs in acute anaphylactic shock. Since the liver is not the only tissue of the dog to contain heparin it was possible that some at least of this heparin may have had its origin in tissues other than the liver. Recently it has been shown in these laboratories [Waters & Markowitz, 1940] that typical anaphylactic shock occurs in sensitized dogs after complete removal of the liver. Applying the discovery of Chargaff & Olsen [1937] that protamine neutralizes the anticoagulant effect of heparin we show that whereas heparin is liberated into the blood of the intact dog in acute anaphylactic shock, no heparin could be detected in the blood of hepatectomized dogs in anaphylactic shock. Preliminary reports of parts of these investigations have appeared elsewhere [Waters, Markowitz & Jaques, 1938; Jaques & Waters, 1940].

METHODS

Sensitization. The dogs were sensitized to horse serum. To the serum 10% potassium alum was added to give a concentration of 1% alum in the final mixture, which was left in the refrigerator for 24 hr. before being injected subcutaneously (0.5 c.c. serum/kg. body wt.). About 6 to 10 weeks later the animals were shocked, under ether or amytal anaesthesia, by the intravenous injection of $1/4$ –1 c.c. serum/kg. body wt. This procedure of treating the sensitizing dose of antigen with alum has been found very effective.

Protamine-heparin titration. Chargaff & Olsen [1937] demonstrated that the anticoagulant effect of heparin on blood, both *in vitro* and *in vivo*, can be annulled by protamine. It had been suggested that the inactivation was due to the formation of an insoluble compound between the heparin and protamine. Experiments in these laboratories show that the inactivation is due rather to the low degree of ionization of this compound of heparin and protamine. We have made use of this property of protamine to determine the concentration of heparin in the blood of dogs in anaphylactic shock. Since protamine itself has a slight anticoagulant action, the amount of protamine required to neutralize heparin in blood is that amount which gives the lowest clotting time of the blood sample. We have found that 1 mg. heparin (Connaught Laboratories) added to dog's blood is exactly neutralized by 1.9 mg. of the protamine used in these experiments, namely Lot no. 5 salmine, prepared by Connaught Laboratories. The amount of protamine required to neutralize a given amount of heparin is not the same with all preparations of salmine protamine. Thus, in earlier studies it was found that 1 mg. heparin was

neutralized by 3 mg. of protamine. It has also been found (see below) that 1 mg. of protamine (lot no. 5) neutralized a different amount of beef heparin from that of the dog.

To six small test tubes (8 mm. diameter), placed in a bath at 37° C., varying amounts of protamine in isotonic saline were added, and the volume made up to 1/2 c.c. with saline. The amounts of protamine usually employed were 0.5, 0.1, 0.05, 0.02, 0.005 and 0.0 mg. About 4 c.c. of blood from the 'shocked' animal were removed from the femoral vein by a syringe moistened with isotonic saline. The first and last 1/2 c.c. of blood in the syringe were discarded. One half c.c. of blood was added to each of the protamine tubes, which were mixed by inverting. The tubes were examined every 1/2 min., and the clotting times noted. The tube with the lowest clotting time indicated the end-point of the titration, but the clotting times of the other tubes were also considered. The manner in which these other tubes were used to obtain a more accurate end-point may be made clearer by means of an example. Thus, if the clotting times with 0.10, 0.05, 0.02 mg. protamine were 10, 7 and 15 min. respectively, it is evident that the end-point was that given by 0.05 mg. protamine. If, however, the clotting times had been 10, 7 and 8 min., the end-point was considered to be nearer 0.02 mg. than 0.05 mg. protamine and would therefore be recorded as 0.02+ mg. protamine. Much smaller concentrations of protamine were used to titrate normal blood of animals before shock. The amounts were 5, 3, 2, 1, 0.5, 0.0 μ g. Occasionally, a slight lowering of the clotting time with these amounts of protamine was noted, but we do not regard the method, as used by us, as sensitive enough to detect any heparin present in any normal blood examined. It has been our experience that 'shock' blood may be stored in the refrigerator for some hours and gives substantially the same heparin value as when titrated with protamine immediately after removal from the animal.

RESULTS

Isolation of heparin from "shock" blood

The methods developed by Charles & Scott [1933] for the isolation of the crystalline barium salt of heparin from beef tissue have been used as the basis of our isolation of heparin from the blood of dogs in anaphylactic shock. Certain drastic modifications in the earlier steps of these methods which we considered advisable when working with blood were made, but these efforts, which need not be detailed, were completely unsuccessful. Subsequently, we were able in one experiment to isolate a few mg. of

crystalline heparin from blood taken from dogs in anaphylactic shock. The accompanying photomicrograph (Fig. 1) shows the crystals to be indistinguishable from those isolated from beef tissues by Charles & Scott. With the experience thus gained we attempted to make as quantitative an isolation as possible of the material from "shock" blood. Samples of the blood were first titrated with protamine solutions to determine the heparin content. The details of this isolation are as follows.

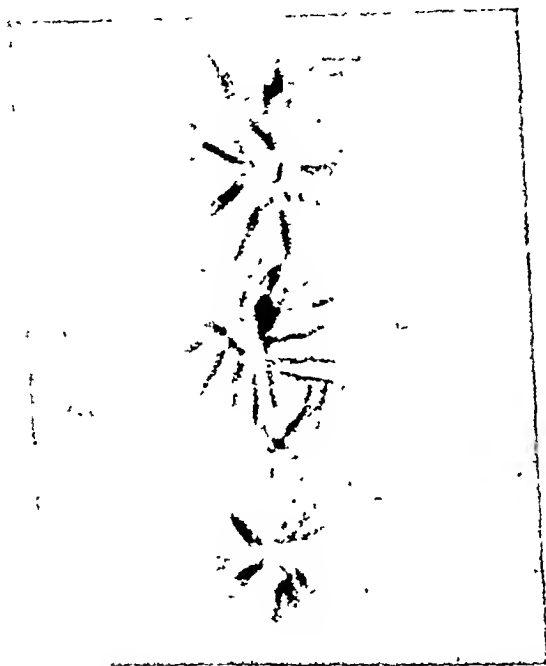


Fig. 1. Crystals of the barium salt of heparin isolated from the blood of a dog in acute anaphylactic shock. $\times 750$.

Dog B. 19.9 kg. Sensitization period 6 weeks, fasted overnight, anaesthetized with amytal. Abdomen opened by midline incision. Seventeen c.c. horse serum injected into splenic vein. Shock was immediate and profound, as can be seen from the accompanying tracing of the arterial blood pressure (Fig. 2). The liver was much engorged and very dark in colour—conditions described as "typical" of anaphylactic shock in the dog. The liver was removed, and the blood which drained from it was collected and added to the blood collected from the abdominal cavity. Blood collected = 945 c.c.

Dog R. 18.5 kg. Sensitization period 8 weeks. It received 15 c.c. horse serum. This experiment was an exact duplicate of the first. Blood collected = 1050 c.c.

Samples of both bloods were titrated with protamine; both bloods required 0.04 mg./0.5 c.c. blood to give the shortest clotting time. The bloods were stored, for convenience, in the refrigerator for about 5 hr., at which time each was separately extracted with an equal volume of 2% NaOH and 1/7 vol. of saturated $(\text{NH}_4)_2\text{SO}_4$ solution, as in the Charles & Scott procedure. The bulky precipitates were separated on Buchner funnels and each washed twice with 150 c.c. water containing 6 c.c. 2% NaOH and 15 c.c. saturated $(\text{NH}_4)_2\text{SO}_4$ solution. The filtrates from the two bloods were then combined and stored in the refrigerator overnight. The following morning a small amount of sediment was removed by decantation and centrifugation. Cold sulphuric acid (conc. H_2SO_4 diluted with an equal volume of water) was added to give a pH of about 2.5. The resulting precipitate was collected, washed with acidulated water, extracted at room temperature with 95% alcohol for 24 hr., then with ether for a few hours. This partially defatted material was suspended in water, dissolved by the addition of 4% NaOH and dialysed against running water for 24 hr. in cellophane tubes. Tryptic digestion of this solution was carried out at pH 8.0 at 40° C. The pH was continuously adjusted by the addition of 2% NaOH. When digestion was complete the material was stored overnight in the refrigerator. Some insoluble material was removed the following morning by long centrifugation, washed with a little alkaline water, and the combined supernatant fluids (about 400 c.c.) concentrated to 85 c.c. at 40° C. This solution was acidified (to litmus) with hydrochloric acid. A small quantity of sodium chloride was added, then 2½ vol. of absolute alcohol. After standing in the refrigerator overnight the precipitate was collected on the centrifuge, washed with acetone, and finally extracted for about 4 hr. with boiling

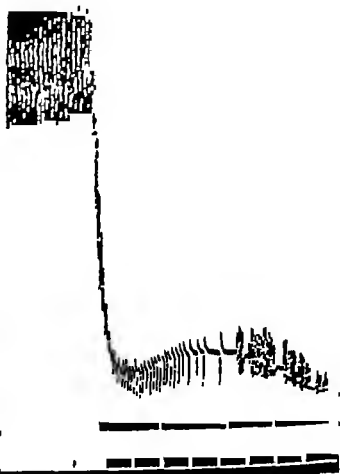


Fig. 2. Blood-pressure tracing of dog, under amylal anaesthesia, in acute anaphylactic shock. Femoral artery pressure before shock 147 mm. Hg (mean). Serum injection into splenic vein. Time intervals 1 min.

acetone. The acetone was removed and the dried material weighed (≈ 3.45 g.). This powder, which assayed at 3.75 units/mg. by the Howell method, was well stirred in 75 c.c. of 5% Na_2CO_3 solution and finally heated to 70°C . The insoluble material was removed on the centrifuge and once washed with 15 c.c. of 4% Na_2CO_3 solution. The clear solution was acidified (to litmus) with glacial acetic acid, warmed and treated with some activated charcoal. The pale yellow filtrate obtained was shaken for a few minutes with a small amount of Lloyd's reagent, and again filtered. To the perfectly clear filtrate 2 vol. of absolute alcohol were added. The precipitate was removed on the centrifuge, washed with alcohol and ether, and dried. It weighed 180 mg. and assayed at 75 units/mg. by the Howell method. The powder was well stirred in 1 c.c. alkaline water at 65°C . and treated with 0.3 c.c. 20% $(\text{NH}_4)_2\text{CO}_3$. The relatively bulky precipitate was washed and the supernatant solutions were combined, acidified with acetic acid, diluted with water to about 6 c.c. and treated with nearly 2 c.c. 10% barium acetate solution. The precipitate was removed by long centrifugation. One c.c. of the perfectly clear solution was taken for trial crystallizations of the barium salt by the addition of glacial acetic acid. The remaining solution was then warmed to 65°C . and 1 c.c. glacial acetic acid added. The solution was left to cool very slowly to room temperature. The following morning the crop of crystals was separated on the centrifuge. In appearance they closely resembled crystalline beef heparin, and appeared to be free of amorphous material. They were washed with alcohol and ether, then air-dried. This batch of crystals constitutes fraction I and weighed 21.3 mg. The mother liquor without further treatment was placed in the refrigerator overnight and a second crop of crystals (fraction II = 9.8 mg.) removed the following day in like manner. To the mother liquor, after the second crop of crystals had been removed, $1/4$ c.c. 10% barium acetate solution was added, and the solution returned to the refrigerator. The following day a third crop of material (fraction III = 5.6 mg.) was collected.

TABLE I. Isolation of heparin from blood of dogs in anaphylactic shock

Stage	Weight	Potency	Total potency
Blood	200 l.	0.04 mg./0.5 c.c. ≈ 8.7 units/c.c.	150 mg. protamine ≈ 77 mg. heparin
After acetone extraction	3.4 g.	3.75 units/mg.	$\approx 18,000$ units
After Lloyd's reagent	0.180 g.	75 units/mg.	12,700 units
Isolated as crystalline barium salt	36.7 mg.	240 units/mg.	13,500 units
Samples removed for trial crystallizations			8,600 units
Samples removed for assays			1,600 units
			<u>300 units</u>
		Heparin accounted for	10,700 units

The amount of active material in the collected blood, as shown by protamine titration, and the amounts present at successive stages of the isolation, including the final crystalline material, are recorded in Table I. The assays of heparin were made by the method of Howell as modified by Charles & Scott [see Jaques & Charles, 1940]. The excellence of the yield of heparin in crystalline condition from the blood, as judged by the preliminary protamine titrations, may perhaps be better than could legitimately be expected from such methods. But it seems reasonable to conclude from these results that heparin is the only anticoagulant substance liberated in anaphylactic shock.

The three crops of crystals isolated from the blood were assayed on several occasions against the Connaught Laboratories' standard preparation of Charles & Scott's crystalline barium salt (100 units/mg.). Three methods of assay have been used; that of Howell (as modified by Charles & Scott), that of Fischer & Schmitz [1932], and another, in which thrombin, as prepared by Mellanby, was used with oxalated beef blood [Jaques & Charles, 1940]. The results of the Howell assays are given fully in Table II. The importance of the order in which the samples are

TABLE II. Potency of heparin isolated from "shock" blood and from dog liver (Howell's method)

From "shock" blood			From dog liver	Date of assay
Fraction I (21.3 mg.)	Fraction II (9.8 mg.)	Fraction III (5.6 mg.)		
—	213	—	—	24. xi. 39
250	275	200	—	25. xi. 39
275	—	—	—	27. xi. 39
225	225	225	—	29. xi. 39
—	—	—	250	16. i. 40
—	—	—	250	17. i. 40
—	—	235	—	21. xii. 39
—	250	225	250	24. i. 40
—	225	238	238	25. i. 40
Av. values	250	238	225	247

All solutions were assayed against the Connaught Laboratories' standard preparation of Charles & Scott's crystalline barium salt (100 units/mg.). The same cat was used for all assays on any one day. Solutions of fractions I, II and III were mixed with blood in this order. On 24. i. 40. Order of sampling: fraction II, liver, fraction III. On 25. i. 40. Order of sampling: fraction III, liver, fraction II.

compared will be evident from the table. There is no significant difference between the potency of the three crops of crystals. This we regard as strong evidence of the homogeneity of the heparin liberated into the blood in acute anaphylactic shock. It will be seen that the heparin isolated from the blood of these dogs in anaphylactic shock is more potent than

that isolated from beef tissues. The value as given by the Howell method of assay is about 2.5 times that of beef heparin.

Using the same methods of isolation we have obtained a crystalline preparation of barium salt of heparin from normal dog's liver. It will be seen in Table II that this heparin isolated directly from liver has the same potency as that isolated from the blood. We have also isolated crystalline heparin from skeletal muscle of the dog. It too has the same potency. The actual values obtained in the assays were 285, 225, 237 and 250, giving an average value of 250 units/mg. It thus appears that heparin isolated from canine tissue is different from that isolated from beef tissue.

The relative potency of beef and dog heparin depends upon the method of assay, or even the conditions of the different methods, and this serves further to show essential quantitative differences in the behaviour of these two substances. Thus an average value of 200 units mg. for dog heparin (in comparison with 100 units mg. for the standard beef heparin) was obtained when the anti-kinase effect on chicken plasma (the Fischer & Schmitz [1932] assay) was used, while the average value was 140 units/mg. when the anti-thrombin action of the heparins was compared. With all methods of assay, variations in relative potency were observed, which were evidently due to changes in the clotting system employed. Thus, with the anti-thrombin method of assay the relative potency of the two types of heparin varies considerably with the age of the oxalated beef blood used. From these results of the various assays it is apparent, therefore, that the two heparins exert different effects at various stages of the clotting system.

These findings provide an explanation for the rather curious fact that the crystalline heparin obtained by Charles & Scott (from beef tissue) was no more potent than the amorphous preparation of Howell. It will be recalled that Howell obtained his heparin from dog liver.

Recently Jorpes & Bergström [1939] have claimed that there are several beef heparins. Charles & Todd [1940] reaffirm the earlier conclusion of Charles & Scott [1936] that the crystalline barium salt isolated from beef tissue is homogeneous. The heparin we have isolated from different tissues of the dog also appears to be homogeneous.

In these isolations of heparin we have followed the customary practice of allowing the minced tissues to autolyse for about 20 hr. at 25° C. We have also succeeded in isolating heparin, though not in such good yields, from both lung and liver tissue without any preliminary autolysis. Presumably the same result would be obtained with other tissues containing heparin.

Attempted isolation of heparin from normal dog's blood

On two occasions about 4 l. of blood were collected from dogs with clotting times within the normal range. The dogs were bled out under anaesthesia and the blood received directly into an equal volume of 2% NaOH. Employing the same methods as for the isolation of heparin from "shock" blood, we were unable to obtain any heparin. The very slight apparent activity (less than 0.6 units/mg.) of the ultimate products (70 mg. in one experiment, 240 mg. in the other) is probably due to its salt content. This interpretation may also apply to several earlier reports of successful isolation from normal blood of material weakly anticoagulant and hence considered to contain heparin.

Protamine titrations of blood taken from dogs in anaphylactic shock

The blood samples were obtained, and titrated with protamine as described above. It should be mentioned that in these experiments the clotting times of the successive blood samples were not necessarily reduced exactly to the pre-shock value, but were reduced always to a value of less than 10 min. The end-point was thus more strictly the smallest amount of protamine giving the shortest clotting time. Typical results of these experiments are given in Table III. It will be seen that the heparin

TABLE III. Protamine titration of blood samples taken from dogs in anaphylactic shock

Time after "shock" dose of antigen (min.)	Protamine* (mg./0.5 c.c. blood) to reduce clotting time to lowest value					
	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5	Exp. 6
Control (pre-shock)	0.0005	0.0001	0.0001	—	0.0001	0.0001
3	0.005	0.005	0.005	0.009	0.01	—
6	—	0.02	0.02	0.015	0.03	0.0002
14	0.02	0.01	0.02	0.02	0.02	—
22	0.05	0.02	0.02	0.025	0.04	0.0001
35	0.02	0.005	0.02	0.03	—	—
45	—	—	0.01	—	—	—
55	0.02	0.005	0.003	0.03	0.03	—
70	0.005	—	—	—	—	—
80	—	—	0.005	0.35	—	—
90	—	0.001	—	—	—	—
110	—	0.001	—	0.03	—	—
130	—	—	0.005	—	—	—
Degree of shock	Fairly severe	Moderate	Moderate	Fairly severe	Fairly severe	No shock

* Dog heparin 1 mg. = 2.1 mg. protamine. Dog heparin 1 mg. = 250 units (Connaught). Therefore to calculate approximate heparin concentration (units/c.c. blood) multiply above protamine figures by 250.

output varies considerably from one animal to another. From some animals, the blood sample with the maximal content of heparin coagulated

within 2 hr. of removal from the blood vessel when kept at 37° C., while at other times the samples remained fluid for more than 48 hr. The maximal heparin value we have observed was 25 units/c.c. of blood.

It is thus seen that the amount of heparin present in the blood following the administration of the "shock" dose of antigen can be obtained by titration of blood samples in the manner described. In the same way the blood of an animal in peptone shock may be titrated. It is well known that there is a very considerable variation in the extent of the coagulation changes in the dog produced by an injection of peptone. A titration curve obtained from a dog receiving peptone has already been published [Waters *et al.* 1938] and need not be enlarged upon here.

Within 3 min. of administration of the shock dose of antigen there is an appreciable output of heparin, but the maximal concentration occurs some time later than this, in some experiments considerably later. Within a minute of beginning the injection of antigen the arterial pressure begins to fall and rapidly reaches a low value of 25–35 mm. Hg. It might well be that the liberation of heparin occurs very rapidly, but that the blood flow through the liver, the source of the heparin (see below), becomes so sluggish on account of the low blood pressure and the characteristic congestion which occurs in this organ, that the rise in concentration of the heparin in the general circulation is very gradual. The alternative view is, of course, that the liberation of heparin from the hepatic cells continues over an appreciable period of time. We are inclined to favour the former view. It is of interest to compare the time relationships of the liberation of heparin into the blood with those of histamine, as found by Code [1939]. He found the maximum concentration of histamine to occur within 3–10 min. after the injection of antigen.

*Protamine titrations of blood taken from hepatectomized
dogs in anaphylactic shock*

Anaphylactic shock has been demonstrated in sensitized dogs after complete removal of the liver [Waters & Markowitz, 1940]. It will be observed from the data in Table IV that these hepatectomized animals in acute anaphylactic shock differ from the intact animals in the amount of protamine required to return the clotting time to the normal. In the absence of the liver the amount of protamine necessary is extremely small. This we interpret as meaning that there is no liberation of heparin in the shocked hepatectomized dogs. It is concluded that the liver is the only source of the heparin occurring in the blood during anaphylactic shock of the dog.

TABLE IV. Protamine titrations of blood samples taken from hepatectomized dogs in anaphylactic shock

Time after "shock" dose of antigen (min.)	Protamine titrations (mg./0.5 c.c. blood)				
	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5
Control (before shock)	0.0002	0.0005	0.0002	0.0002	0.001
3	0.001	0.0002	—	—	—
5	—	—	0.0005	0.0005	0.001
10	0.0002	—	0.0002	—	0.001
15	—	0.001	—	0.0001	—
25	—	0.0002	0.0005	—	—
30	—	0.001	—	0.0001	—
40	—	—	0.001	—	—
60	—	—	—	0.0001	—
80	—	—	—	—	—

While there was no appreciable difference between the amount of protamine required to lower the clotting time of the blood obtained during anaphylactic shock, yet there was in these hepatectomized dogs an increase in the clotting time of the blood during shock. In one experiment the clotting time increased from 4 to 45 min. The very small amount of protamine required to annul this increase suggests that it is not due to heparin. Investigations in collaboration with Dr E. Fidler indicate that this prolongation of the clotting time may be directly associated with the considerable concomitant decrease in the platelet count.

The heparin content of the liver during anaphylactic shock

As there is no liberation of heparin into the blood of hepatectomized dogs in anaphylactic shock we have concluded that the liver is the sole source of this material when the intact animal is shocked. It should therefore be possible to demonstrate a decrease in the amount of heparin which can be extracted from the livers of the latter animals in comparison with that from normal animals. We have extracted the minced livers by the method of Charles & Scott, and the final product has been assayed by the method of Howell. These results are given in Table V. It will be readily seen that the heparin which was extracted from the livers of the shocked animals is appreciably less than the average amount obtained from the control livers which, it is true, showed some variation. There are a number of possible sources of error in these procedures when applied to "shocked" livers and to normal livers. These have been only partly investigated and will not be enumerated here. While they may necessitate certain corrections to our values we do not think the corrections would be of such magnitude as to invalidate our conclusion that "shocked" livers contain less heparin than normal livers.

TABLE V. "Heparin contents" of normal and "shocked" dogs' livers

Units/100 g. tissue			
Normal units	"Shocked"		Time of removal of liver after "shock" dose of antigen
	Units	Max. heparin titre (units/c.c.) noted in blood	
470	68	12	25 min. (at death of animal)
1900	400	5	120 min.
1450	17	—	30 min.
310	147	5	200 min. (at death of animal)
2300	—	—	—
1920	—	—	—
1990	—	—	—

Changes in the mast cells of the liver

Further support for the conclusion that the heparin present in the blood during anaphylactic shock originates in the liver is obtained by examination of the mast cells of this organ after the shock. Wilander, using a toluidine-blue staining reaction, has provided a convincing demonstration of his assertion that heparin is present in large amount in the mast cells. He has further shown that extensive damage occurs to the hepatic mast cells of dogs suffering from peptone shock [Wilander, 1939]. With the co-operation of our colleague, Dr D. L. MacLean, who, employing the method of Wilander, made histological studies of liver sections, we have been able to confirm Wilander's findings in peptone shock and to extend them to anaphylactic shock.

SUMMARY

1. The anticoagulant substance responsible for the characteristic prolongation of the clotting time of blood of dogs in anaphylactic shock is heparin.
2. Heparin has been isolated in a crystalline condition and in good yield from the blood of dogs in anaphylactic shock.
3. With the same methods, no heparin could be isolated from the blood of normal dogs.
4. By means of a protamine titration procedure, the rate at which heparin appears (and disappears) from the blood in anaphylactic shock has been measured. Typical results are recorded.
5. The heparin obtained from different dog tissues (liver, muscles and from blood during anaphylactic shock) is the same, but is different from heparin isolated from beef tissues. Its anticoagulant potency is 2.5 times greater than that of beef heparin.

6. No heparin is liberated into the blood of sensitized hepatectomized dogs in anaphylactic shock. It is concluded that in the dog in anaphylactic shock the heparin is liberated into the blood from the liver only.

7. The amount of heparin which can be extracted from the liver of dogs in anaphylactic shock is much less than that extracted from normal dog liver.

It is a pleasure to acknowledge our indebtedness to Dr A. R. Charles for his generous advice on the chemical procedures involved in the isolation of crystalline heparin. We also wish to thank Dr James Craigie for the preparation of the photomicrograph.

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SKELETAL CHANGES AFFECTING THE NERVOUS SYSTEM PRODUCED IN YOUNG DOGS BY DIETS DEFICIENT IN VITAMIN A

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I. INTRODUCTION

THE production under controlled conditions of tissues abnormal in structure or function, or in both, and the prevention of the abnormalities by defined alterations in these conditions have sometimes led to the accession of knowledge both to the physiologist and pathologist. The present paper deals with an instance of this type of experiment.

In previous publications detailed descriptions have been given of the widespread degeneration of the central and peripheral nervous systems, especially of young animals brought up on diets deficient in vitamin A and carotene and rich in cereals [Mellanby, 1926, 1931, 1933, 1934, 1935]. Although these degenerative changes were thought at first to be directly due to the destructive effect of vitamin A deficiency on the nervous system, evidence was subsequently forthcoming to suggest that they were indirectly produced and were really due to bone overgrowth in the vicinity of the affected nerves and nerve cells and to the pressure effects resulting therefrom. In particular, a study of the eighth nerve and the labyrinthine capsule [Mellanby, 1938] demonstrated that a dietary deficiency of vitamin A resulted in bony overgrowth of the periosteal layer of the labyrinthine capsule, especially of that part surrounding the internal modiolus, and the evidence strongly suggested that the pressure due to this overgrowth was responsible for the nerve degeneration. At a later date, evidence was also given of bony overgrowth surrounding the optic and trigeminal nerves and of other bones in close proximity to the brain and spinal cord [Mellanby, 1939*a*, *b*]. Although it cannot yet be claimed that this sequence of events fully explains all the widespread

That such reactions may afford the fundamental explanation of the widespread changes in bone is in keeping with the importance of age and rate of growth in determining the rate of development of the bone hyperplasia, for it is in the early stages of life and in places of more rapid growth that osteoblasts and osteoclasts are more numerous and active.

It may be well to add that the effects of vitamin A deficiency here described must be considered in relation to the basal diets given. There is indeed evidence that some modifications of the basal diet, even in the absence of vitamin A, also affect the results, at least in degree if not in kind. This is not surprising in view of the numerous known dietetic factors which influence bone structure and bone growth. In particular, the addition of calcium salts and the presence or absence of vitamin D modify the bone overgrowth in the absence of vitamin A. The results obtained under the conditions described do, however, show that bone growth is affected by vitamin A and that these changes may have notable effects on nerve function and animal behaviour.

II. EXPERIMENTAL METHODS

Litters of puppies from 7 to 10 weeks old were fed on basal diets of the following type: separated milk powder 20 g., cereal (usually white bread) 100-300 g., lean meat 15-20 g., yeast 3-12 g., peanut or olive oil 10 ml., orange or lemon juice 6 ml., sodium chloride 1-2 g., and irradiated ergosterol (vitamin D₂) 1000-2000 international units. This diet, although made up of natural foodstuffs, with the exception of vitamin D₂, is deficient in vitamin A and carotene, there being only a small amount of vitamin A in the lean meat and a trace of carotene in the cereal. The diet is otherwise good, although its calcium content may not be sufficient to allow optimal calcification of bones when growth is very rapid. In such cases a certain degree of osteoporosis, but never rickets, may develop even when vitamin A is present; otherwise the diet allows good growth and healthy development, except for the abnormal changes produced by vitamin A deficiency. In each litter one or more puppies were given an additional supply of vitamin A or carotene; the former usually as mammalian liver fat preparation, but sometimes as cod-liver oil, and the latter as carotene in cabbage or as the pure substance. The form in which vitamin A or carotene is given does not appear to matter, and, so long as sufficient is absorbed from the alimentary canal, normal bones result and the nervous system does not show the characteristic degenerative changes.

Preparation of tissues for examination

At the end of each experiment, the tissues were prepared for histological examination. In order to reduce shrinkage of soft tissues to a minimum, an intra-arterial fixation method was used and in most cases serial sections of the bones were made. The methods of preparation used were largely in accordance with Wittmaack's technique and his solution was generally used for intra-arterial fixation.

III. BONE OVERGROWTH AND ITS EFFECTS ON THE NERVOUS SYSTEM

A. *Skull, brain and cranial nerves*

A comparison of mesial sagittal surfaces of the skulls of two litter mates, one of which had a diet containing much vitamin A and the other a diet deficient in the vitamin, shows some of the regions which are especially subject to bone overgrowth. Diagrams of the cut surfaces are given in Text-figs. 1a and 1b; Text-fig. 1a represents the mesial sagittal section of the skull and brain of a dog brought up on a diet containing much vitamin A (+A dog), Text-fig. 1b of a dog whose diet was otherwise the same except that it was deficient in this vitamin (-A dog). (For the sake of brevity the terms +A and -A dog (or animal) will be used to denote dogs brought up on diets containing, or deficient in, vitamin A respectively.)

It will be noted that the brain of the animal maintained on a diet deficient in vitamin A is more tightly packed into the cranial cavity than that of its litter mate receiving vitamin A. It will also be seen that the bones showing the most overgrowth are those surrounding the cerebellum, medulla oblongata and pons. In particular, all parts of the occipital bone, both above and below the foramen magnum, are greatly enlarged (Text-fig. 1b) as compared with the normal (Text-fig. 1a). Passing forward from the supra-occipital bone, the parietal bone is also much thickened in its posterior part, but this thickening becomes less as it approaches the frontal bone. Similarly, at the base of the skull, passing forward from the hypertrophied basi-occipital bone, the enlargement of the basal portion of the sphenoid, although definite, is not so great as in the occipital bone.

The effect of this overgrowth of bone at the posterior end of the skull is to press on the cerebellum and medulla oblongata and alter their shape. The cerebellum is flattened on its dorsal surface and its posterior surface is indented with the thickened occipital bone just above the foramen magnum. In the particular skull illustrated in Text-fig. 1b, the tentorium cerebelli has become calcified and a wedge of bone separates

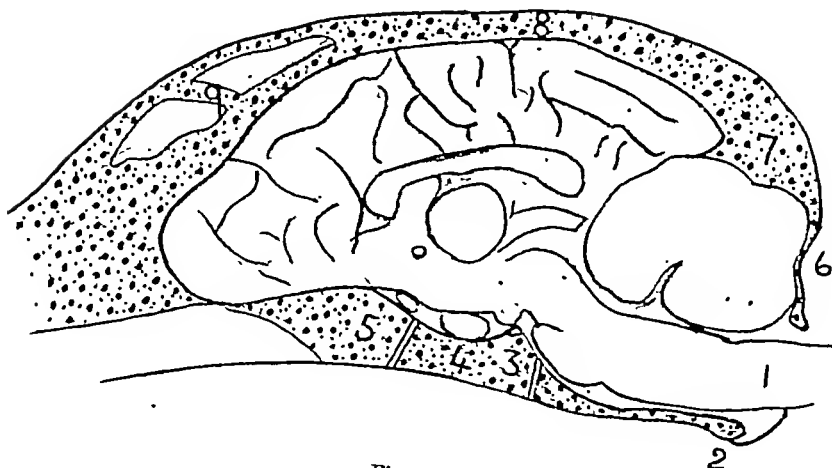


Fig. 1a.

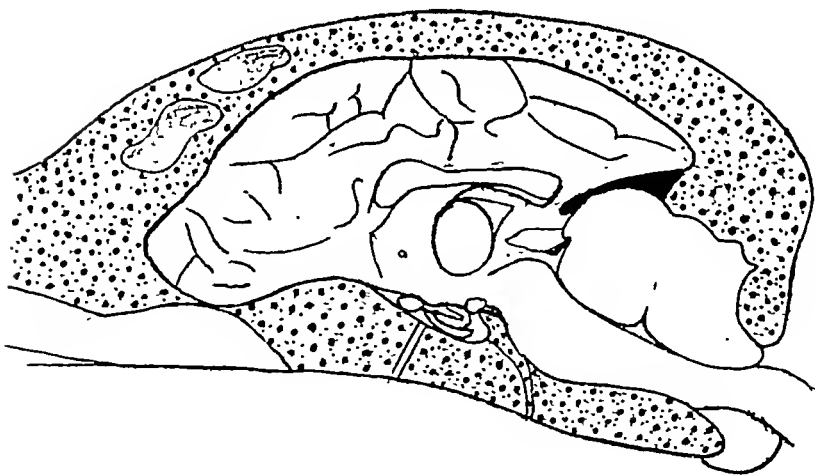


Fig. 1b.

Text-figs. 1a and 1b. Mesial sagittal sections of skulls of +A (a) and -A (b) dogs (bones stippled). Note great increase in thickness of bones forming base of skull, supra-occipital and to a less extent other bones in 1b as compared with those of 1a; also compression of medulla and cerebellum and pushing back of the posterior part of the cerebellum into the foramen magnum in 1b. 1, foramen magnum; 2, basi-occipital; 3, posterior clinoid process; 4, basi-sphenoid; 5, anterior clinoid process; 6, supra-occipital; 7, occiput; 8, parietal; 9, frontal. The calcified tentorium cerebelli is marked black in text-fig. 1b.

the occipital lobe of the cerebrum from the anterior dorsal portion of the cerebellum. Text-fig. 1*b* also shows that the posterior ventral portion of the cerebellum is pressed backwards through the foramen magnum between the occipital bone and the dorsal surface of the medulla oblongata, a space which is normally part of the cisterna magna. This intrusion of the cerebellum into the cisterna magna can be better appreciated in Pl. I, fig. 2*b* (compare Pl. I, fig. 2*a*, the cisterna magna of +A dog).

The medulla oblongata, instead of being cylindrical in that part just ventral to the cerebellum as in Text-fig. 1*a* (+A), is compressed in Text-fig. 1*b*, and the 4th ventricle and the aqueduct of Sylvius are narrowed and reduced in capacity. The overgrowth of the occipital bone surrounding the foramen magnum lessens the area of the aperture through which the posterior end of the medulla passes and must press on this part of the nervous system. Actual narrowing of the dorsi-ventral cross-section of the medulla at this point can be seen by comparing Text-fig. 1*b* (-A) with Text-fig. 1*a* (+A).

In Text-fig. 1*b* there is also a change in the posterior clinoid process at the posterior end of the sella turcica. It is enlarged dorsally and bent forward at its free end. In some experiments this overgrowth has been so great that it has compressed the pituitary body.

While the above are some of the more obvious defects seen in the sagittal sections, removal of the brain reveals other bone overgrowths. Most prominent is the hypertrophy of the petrous portion of the temporal bone. The petrous ridge is normally a fine crest of compact bone, but in -A animals it is a bulbous mass of cancellous bone with a very thin covering of compact bone. This overgrowth must further reduce the space available for that part of the brain contained in the posterior fossa and thereby increase the pressure on the pons, the cerebellum, medulla oblongata and nerves closely related to them. Reference has been made in previous papers to the hyperplasia of this portion of the temporal bone in relation to the 8th nerve and an account has been given of its stretching effect on the nerve and, indeed, of the complete destruction of the nerve in some cases by the occlusion of the passage from the labyrinth to the brain [Mellanby, 1938]. The enlargement of the petrous ridge also partially closes the 5th nerve foramen and compresses this nerve and the gasserian ganglion. When the nerve and ganglion are dissected out, they show a definite constriction where they have passed under and been compressed by the petrous ridge. The increased size of the temporal bone in this position also stretches and pinches the 7th nerve. The 9th, 10th and 11th nerves are trapped between the enlarged temporal and basi-

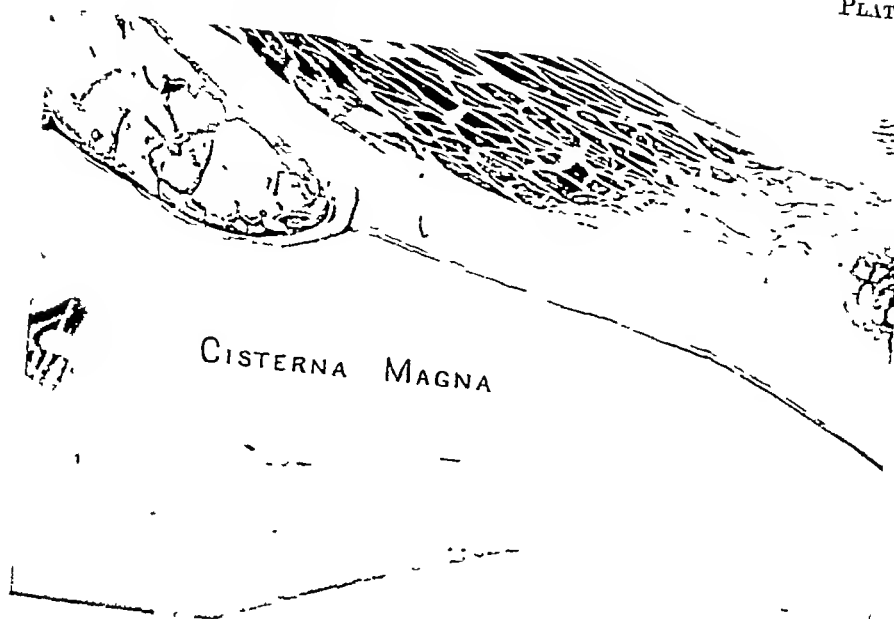


Fig. 2a.

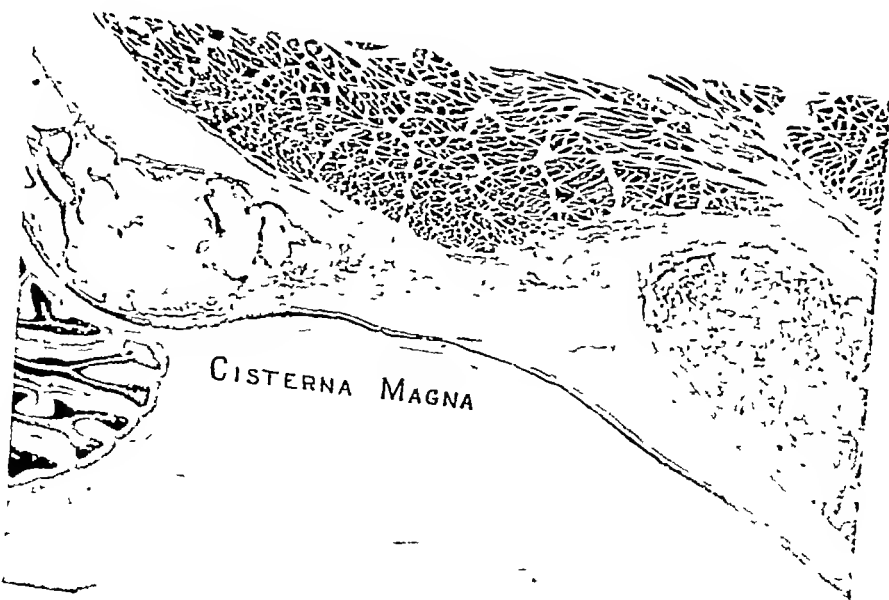




Fig. 3a.



occipital bones in their passage through the jugular foramen. Examination of serial sections through the jugular foramen of -A and +A animals suggests that the glossopharyngeal nerve escapes destructive pressure change, while the vagus and its ganglion and the spinal accessory nerve are probably compressed. The 12th nerve passes directly through the thickened basi-occipital bone; it is elongated in this part of its course and may be compressed. Thus, it will be seen that all the nerves from the 5th to the 12th, with the exception of the 6th, are liable to be directly affected by compression and stretching owing to the overgrowth of the temporal and other bones forming the posterior cranial fossa.

Passing forward in the brain to the middle cranial fossa, the direct pressure of the bone is less, since neither the sphenoid nor the parietal bone shows the great thickening noted in the temporal and occipital bones.

While the bone changes in the neighbourhood of the anterior cranial fossa are not large, especially as compared with those of the posterior cranial fossa, they may nevertheless damage the optic nerve. This is affected in two ways. In the first place, the anterior clinoid process may be enlarged and thus bring about a lateral displacement of the optic foramen which tends to stretch and to bend the nerve. In addition, the optic foramen is narrowed by bone overgrowth and the nerve may be compressed. In advanced cases these effects can be readily seen when the optic nerve is removed from the skull: it is constricted in that part which passes through the optic foramen and it is tortuous and lengthened by the lateral displacement of the foramen. Blindness in calves due to constriction of the optic nerve has been described by Moore, Huffman and Duncan [1935]; also Moore [1939].

There is also enlargement of the cribriform plate, again with a superabundance of cancellous tissue at the expense of its compact bony covering. In the dog the cribriform plate forms a large part of the wall of the anterior cranial fossa. Although the cribriform plate in -A dogs does not appear very abnormal to the naked eye, it will be shown in a later publication that the overgrowth may be disastrous to the branches of the olfactory nerve which pierce the plate, for in some cases many of the nerve fibres are undoubtedly compressed and destroyed. While it is true in general that overgrowth in the frontal bone is small as compared with that in some other bones in the skull, thickening of this bone may sometimes take place at the expense of the frontal sinus, which is reduced in size. Beneath and slightly posterior to this sinus the frontal bone may occasionally be seen bulging into the anterior cranial fossa, whereas in the normal dog this area is usually concave.

Only a brief reference to the condition of the cranial nerves has been given here. Serial sections through the skull bones and the nerves have been made and will be described elsewhere. These confirm that in many cases there is actual compression and stretching of the nerves by bone overgrowth and this overgrowth may well account for most of the degenerative changes in the nerves.

The following measurements of skulls illustrate some of the main overgrowths which directly affect the brain. They represent measurements of comparable regions of the skull bones of two dogs (litter mates) on vitamin A rich and vitamin A deficient diets respectively. (See Text-figs. 1a and 1b.)

Bone	+ Vitamin A dog mm.	- Vitamin A dog mm.
Foramen magnum (dorsi-ventral) (1)	15.8	12.5
Basi-occipital (2)	4.2	8.5
Posterior clinoid process (3)	9.0	12.0
Basi-sphenoid (4)	3.8	6.0
Anterior-clinoid process (5)	10.0	13.0
Supra-occipital (6)	3.0	6.9
Occiput (7)	11.5	18.0
Parietal (8)	4.0	6.0
Frontal (9)	5.0	6.0

These figures again prove that with a deficiency of vitamin A there is great hypertrophy of the bones surrounding the posterior fossa and a smaller overgrowth of those anterior to this fossa. The reduction in the size of the foramen magnum is also apparent.

B. *Changes in intracranial pressure*

That the pressure on the brain is increased in certain places is undoubted for, as shown above, at least in the posterior cranial fossa, the brain is actually deformed by the bone overgrowth. The question arises as to whether there is a general increase in intra-cranial pressure and, if so, whether it is equal in all parts. In this connexion a number of points must be considered. In the first place, the overgrowth is proportionately much greater in the bones surrounding the hinder part of the brain. If there were no obstruction between the posterior and middle cranial fossa, the increased pressure of the hypertrophied bones on the pons, cerebellum and medulla oblongata would undoubtedly be spread over the whole brain. This spread, however, is probably hampered on the dorsal aspect by the tentorium cerebelli; in some of the - A animals this obstruction is more effective because, instead of being membranous as in normal animals, the tentorium is partially calcified. The cerebrospinal fluid, however, will presumably tend to distribute the pressure via the basa

cisternae and subarachnoid space, which are continuous between posterior and middle fossa.

Direct observation indicates that the excessive bone overgrowth round the posterior fossa specially affects the 4th ventricle, the cisterna magna and their contents (Text-fig. 1*b*). The capacity of these spaces is reduced and the pressure of the cerebrospinal fluid in them might be expected to be increased. An attempt was made to record the pressure of the cerebrospinal fluid in the cisterna magna of some of these experimental animals. There is no difficulty in doing this in dogs whose diets contain vitamin A, but in the animals receiving the deficient diet the bone overgrowth makes it difficult to reach the cisterna, the reduced size of which (Pl. I, figs. 2*a* and 2*b*) also increases the risk of damaging the medulla, so that it is often difficult to obtain samples uncontaminated by blood. In one or two of the animals on the A deficient diets there appeared to be little or no cerebrospinal fluid in the cisterna magna. This point will receive consideration later. The pressure of the fluid in the cistern has been successfully recorded in four - A and four + A dogs. The average pressure in the - A group was 100 mm. of water, compared with 58 mm. in the + A group.

If, therefore, the foramina of Luschka are still patent there should be a similar increase in pressure in the 4th ventricle, the subarachnoid space surrounding the brain and cord and in the other cisternae of the base of the skull, namely the cisterna pontis, the cisterna interpeduncularis and the cisterna chiasmatis, with all of which the cisterna magna is in direct communication. The mere fact that there is usually fluid in the cisterna magna probably indicates that, even in dogs fed on diets deficient in vitamin A over long periods, the foramina of Luschka are open, because it is generally accepted that the cerebrospinal fluid is secreted in the choroid plexus, that there is very little of this tissue in the cisterna magna, and what there is protrudes from the fourth ventricle through the foramina of Luschka. From the dog, the foramen of Magendie, another passage of communication from the lateral ventricles to the cisterna magna in the human being, is absent and as far as is known there is no other outlet, although it has been suggested that there is a connexion between the lateral ventricles and the cisterna interpeduncularis. Complete closure of the foramina of Luschka by pressure from bone overgrowth might be expected, therefore, to prevent the passage of fluid from the brain ventricles to the subarachnoid space, cisternae, etc., including the cisterna magna, and what fluid was present in the latter space would tend to be absorbed. In those cases, referred to above,

where no evidence of cerebrospinal fluid in the cisterna magna of -A dogs could be obtained by puncture, it is possible that the foramina of Luschka were occluded. However, no certain evidence of complete closure of these foramina has so far been obtained. On the other hand, there is no doubt that both the 4th ventricle and the foramina of Luschka may be greatly reduced in size in -A dogs. This can be observed by comparing Pl. II, figs. 3*a* and 3*b*. It can also be seen how closely packed is the choroid plexus in the 4th ventricle and foramina of Luschka of a -A dog (Pl. II, fig. 3*b*) as compared with the same tissue in the 4th ventricle of a +A animal (Pl. II, fig. 3*a*).

Let us now turn to the other brain ventricles, the 3rd and lateral. If the aqueduct of Sylvius were completely occluded, it is known, from the experiments of Danby & Blackfan [1914, 1919] on the dog that a condition of internal hydrocephalus would result. By analogy, it would be expected that if the pressure due to bone overgrowth in these experiments was sufficient to occlude the aqueduct of Sylvius the cerebrospinal fluid would continue to be secreted by the choroid plexus; and since this fluid could not pass into the 4th ventricle and thence to the cisternae and the subarachnoid spaces to be absorbed, the accumulation would increase the pressure in the lateral ventricles, expand them and cause an internal hydrocephalus.

What, then, is the state of the lateral and 3rd ventricles in these -A dogs? This can be seen in Pl. III, fig. 4*b* and compared with the same spaces in a normal litter mate in Pl. III, fig. 4*a*. A condition resembling internal hydrocephalus is present in Pl. III, fig. 4*b*, and both the lateral and 3rd ventricles are expanded. The expansion is not, however, of the same order as that produced by Danby & Blackfan in their dogs, in which the aqueduct of Sylvius was completely occluded by direct surgical methods. It is possible, therefore, that the internal hydrocephalus produced in the diet experiments is not due to the complete occlusion of the aqueduct of Sylvius, but that the increased pressure in the 4th ventricle and the cisterna magna is communicated through the aqueduct of Sylvius to the 3rd and lateral ventricles and expands them.

This, however, is a complicated problem, for it is necessary to consider not only the actual direct pressure effects of bone overgrowth on different parts of the brain and cerebral fluid and the patency of the aqueduct of Sylvius and the foramina of Luschka, but also the relative rate of secretion of cerebrospinal fluid by the choroid plexus and its absorption by the cells surrounding the various ventricles and the subarachnoid spaces. Nevertheless the foregoing examination shows that

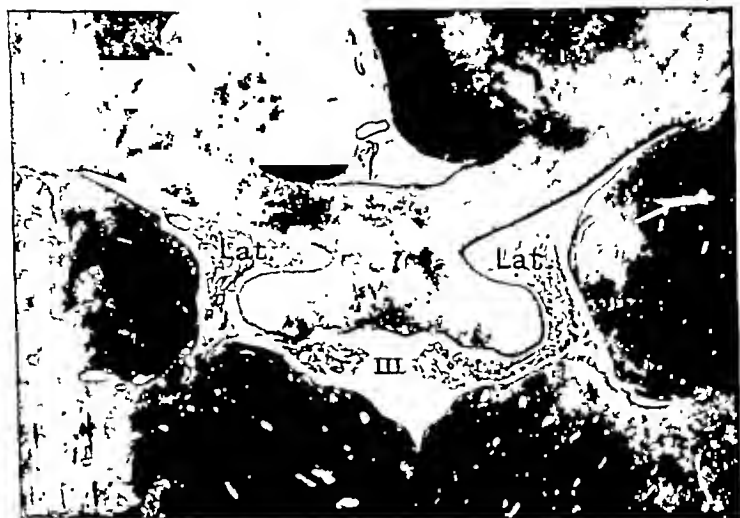


Fig. 4a.



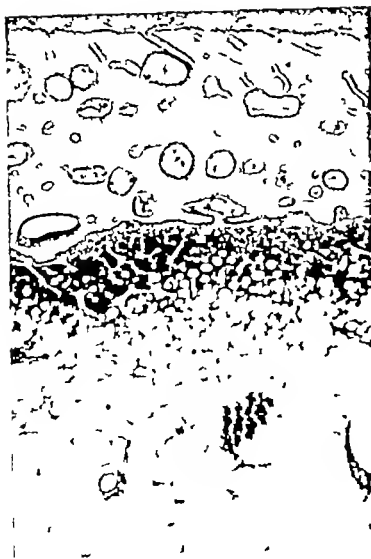
Fig. 4b.



Fig 5a



Fig 5b



there is often a large increase of pressure in the cerebrospinal fluid of -A dogs and that, associated with this, there is

(1) compression and reduction in size of the 4th ventricle, cisterna magna and other cisternae at the base of the brain;

(2) a condition of internal hydrocephalus with expansion of the lateral and 3rd ventricles.

Whether there is ever complete occlusion of the aqueduct of Sylvius and the foramina of Luschka, either temporarily or permanently, is not known, but the evidence is rather against it, although there is certainly definite narrowing of these passages. The results of examination indicate that in these -A dogs the whole brain and probably the spinal cord is subjected to increased pressure. The cerebrospinal fluid mechanism tends to diffuse this rise of pressure to all parts of the central nervous system, but whether it succeeds in doing this in severe cases of -A deficiency or whether it fails in such instances because of a break in continuity of the fluid is not known. Where the anatomical changes above described are great, it is undoubted that the total capacity of the spaces containing cerebrospinal fluid is much reduced as compared with that of the normal animal. It might be expected therefore that the one known function of this fluid, namely to distribute pressure evenly and thereby to lower it at local points of excessive pressure, as for instance in the posterior fossa of the cranium, is impaired in -A animals.

C. Vertebral column, spinal cord and spinal nerves

The kind of differences observed in the shape and texture of the bones of the skull of -A and +A dogs can be seen also in the bones of the vertebral column. All delicacy of outline present in these complicated structures when normal disappears in the vitamin A deficient animals and the bones become swollen and coarse. There seems to be little or no increase in the overall dimensions of each vertebra, but all the processes, including the arches and articular processes, are thickened to a greater or less extent. For instance, the wings of the atlas vertebra in one animal which received vitamin A were 2.2 mm. thick, whereas the thickness of the same region in its litter mate not receiving vitamin A was 4.95 mm. Instead of finely moulded edges (Pl. IV, fig. 5a), bulbous protuberances are usually found (Pl. IV, fig. 5b). It is unnecessary to describe these anatomical changes in individual vertebrae here, but their effect on the spinal cord and the spinal roots calls for some consideration. When the spinal cord is exposed by the cutting through of the vertebrae, it can be seen at once to be much more closely packed into the spinal

canal in animals which were fed on $-A$ diets than in those with a sufficiency of the vitamin. A fatty material can also be seen in the former closely enveloping the cord between the dura and the vertebrae. This probably does not indicate an actual increase in fat in this position, but may be the result of the reduced size of the space.

Some of the changes in the bones and tissues immediately surrounding the spinal cord are represented in Pl. V, figs. 6*a* and 6*b*, which are photomicrographs of sections through the intervertebral discs of the 5th cervical vertebra of litter mates, one a $+A$ animal (Pl. V, fig. 6*a*) and the other a $-A$ animal (Pl. V, fig. 6*b*). One of the difficulties in this work is that the bone overgrowths, especially in complicated structures, make histological examination of identical positions difficult or impossible. For instance, Pl. V, figs. 6*a* and 6*b* are both roughly through the centre of the dorsal root ganglion, but owing to the bone overgrowth, the relative positions of other structures are altered and are therefore not necessarily truly comparable. This difficulty is partially overcome in practice by examining the serial sections which have been made in most of these experiments, but it is not possible to publish the necessary photomicrographs to show this. It will be seen, however, that, although there is not much change in the total area covered by the cross-section of the bones in these two dogs, the internal space traversed by the spinal cord, its membranes and nerves is greatly reduced in the case of the $-A$ dog. An idea of the size of the changes in the various areas of such cross-sections can be obtained from the following figures, which represent a series of measurements made by weighing paper which just covered each part of the section and estimating each area by the weight of paper. The animals were injected with Wittmaack's solution, which tends to prevent shrinkage, so that the figures obtained probably approximate to those in life.

Areas of various regions of vertebral cross-sections
(5th cervical) of $+A$ and $-A$ dogs

Regions	Sq. cm. actual area		Percentage of area	
	$+A$	$-A$	$+A$	$-A$
Spinal cord	0.338	0.336	8.05	8.01
Space between cord and dura	0.212	0.160	5.05	3.81
Space between dura and bone	0.668	0.384	15.9	9.15
Intervertebral disc and body of vertebra	1.428	1.547	34.0	36.88
Lateral portions of vertebra	1.371	1.614	32.65	38.48
Whole section of vertebra	4.200	4.195	100.0	100.0

These figures show (1) that the total area covered by the cross-sections of the comparable bones is not significantly altered in the $-A$

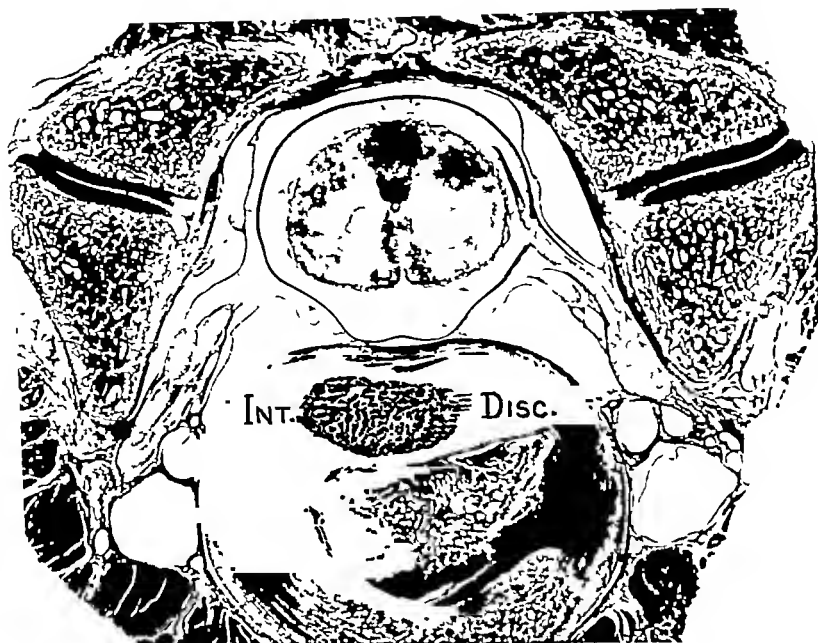


Fig. 6a.

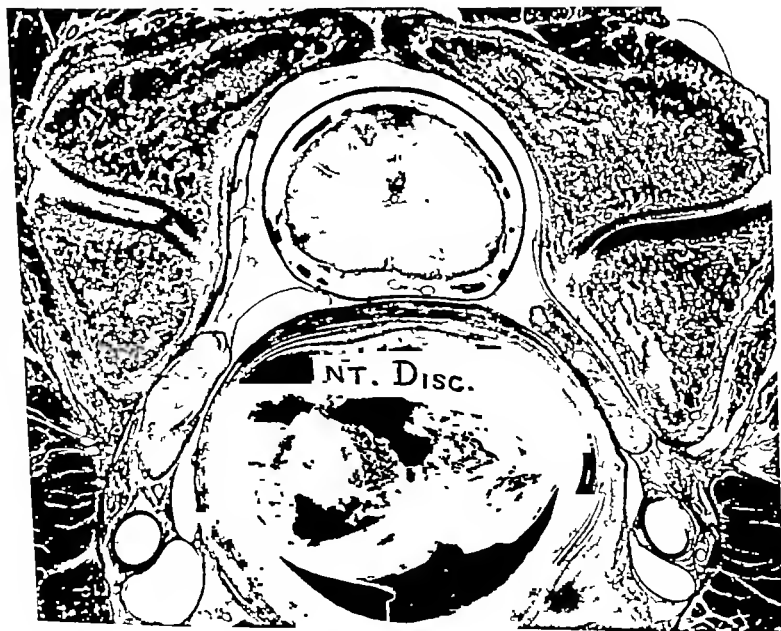


Fig. 6b.

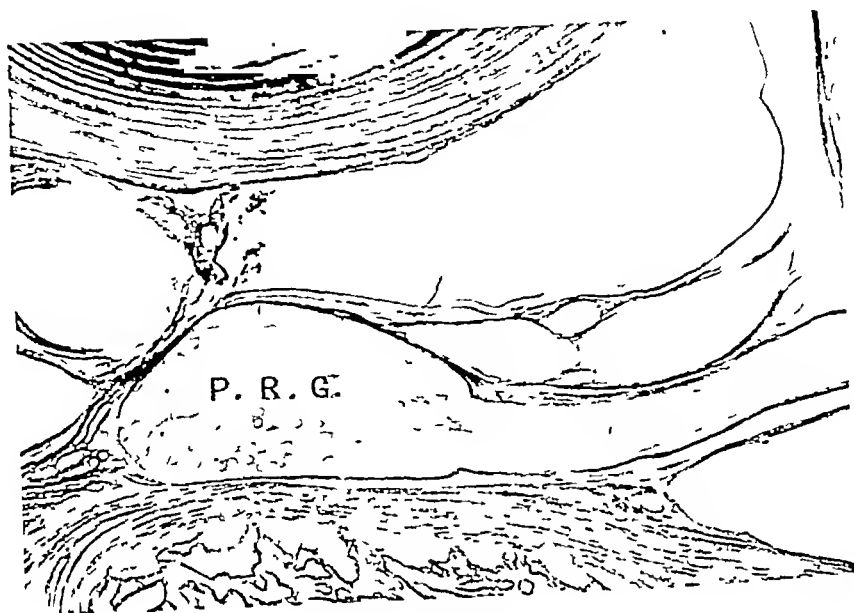


Fig. 7a.

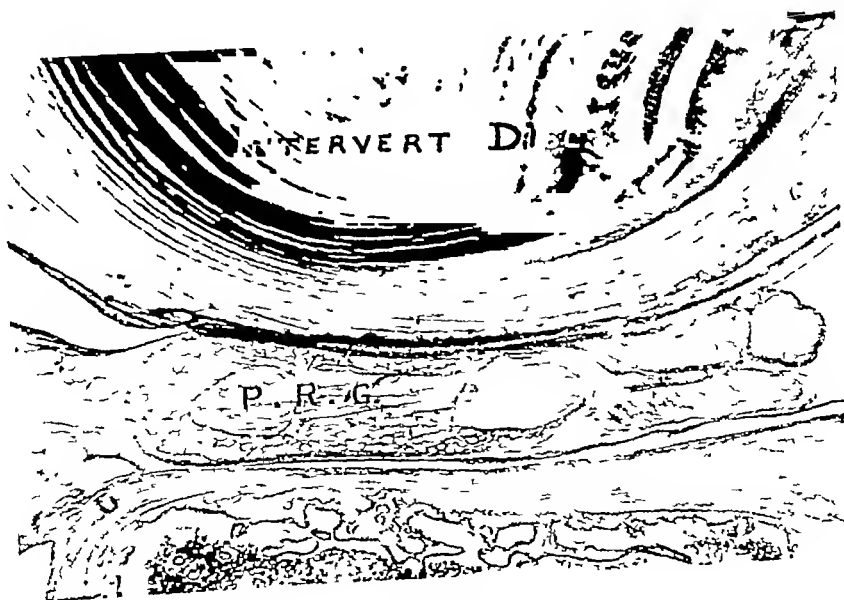


Fig 7b

animal, i.e. the vertebrae are not generally larger but are only locally thickened; (2) that the area of the lateral portions of the vertebra is increased in the -A dog; (3) that the main change in the -A dog is the reduction in space between the dura and the bone and to a less extent between the spinal cord and the dura.

Examination shows that one reason for the reduction in size of the spinal canal is the inward growth of the lateral mass of the vertebra, thus encroaching on the spinal canal.

The change in position of the vertebral artery and vein relative to the body of the vertebrae can also be seen. In the -A animal (Pl. V, fig. 6b) these vessels are placed more dorsally in relation to the intervertebral disc than those in the +A animal (Pl. V, fig. 6a).

As regards the space surrounding the spinal cord, it will be seen that (1) the reduction of the space between the dura mater and the cord is general but is greater on its ventral side, and the slight swelling in this position in the normal animal (Pl. V, fig. 6a) is absent from the -A animal (Pl. V, fig. 6b); (2) there is a great reduction in the size of the spaces between the body and the wings of the vertebrae for the passage of the spinal nerves. In fact, on the left side of the -A dog (Pl. V, fig. 6b) compression of these nerves and especially of the posterior root ganglion can be seen between the wings and the body of the vertebra: this state of compression, in comparison with the free space occupied by the corresponding nerves and ganglion in the normal animal, can be better appreciated in Pl. VI, figs. 7a and 7b, which are higher power photomicrographs. There is also some lengthening of the spinal nerves during this passage through the spinal canal of -A dogs, because the nerve roots pass more obliquely across the canal towards the periphery than in the +A animals.

The pressure exerted by the bones on the spinal nerves and the posterior root ganglion in the animals fed on vitamin A deficient diets is undoubtedly largely responsible for the degenerative changes previously described in these nerves [Mellanby, 1933, 1934, 1935], although at the time this was not appreciated. Attention, however, was drawn to what at that time seemed a curious fact, namely, that the anterior roots in the spinal canal central to the posterior root ganglia generally escaped degenerative changes, whereas the nerve fibres of the posterior roots in the same position were often degenerate. The reason for this difference in distribution of nerve degeneration now seems clearer. The nerve cells of the posterior root ganglion are often damaged by the effects of pressure and their axis cylinders, both peripheral and central to the ganglion,

therefore degenerate. On the other hand, even if the anterior roots are destroyed by pressure at the same spot, the fibres central to the lesion will escape degeneration since their ganglionic origin is in the anterior horns of the spinal cord. The fibres of the anterior spinal nerves peripheral to the compression may, however, be destroyed. There is certainly great degeneration in the sciatic nerve and other peripheral nerves of spinal origin, but whether the efferent as well as the afferent fibres are affected is not known.

Another observation made in the earlier work was that all the spinal nerves in a given — A dog did not suffer equally. Even at the same level. of the vertebral canal, sometimes the spinal nerves on one side escaped destructive changes while those on the other side suffered. The reason for this difference can now be understood. In Pl. V, fig. 6*b*, for instance, it will be seen that the spinal nerves and posterior root ganglion of the right side (left side in Pl. V, fig. 6*b*) are not subjected to the same pressure effects as those of the left, so that the latter are more destroyed than the nerves of the right side.

It may be asked whether the bone overgrowth and malformation in the vertebrae of — A dogs explain all the degenerative changes seen in the cord. The bone abnormalities will certainly account for the degeneration of fibres having their origin in the posterior root ganglia and entering the cord. It has also been explained that the bone changes in the upper cervical region and in the bones surrounding the medulla oblongata are particularly large, so that the pressure effects in this region are great and may well be responsible for the increase in the number of degenerated fibres in the nervous system of these parts. Direct pressure on the cerebellum, medulla and upper cervical region of the cord may bring about the degenerative changes of the anterior and posterior cerebellar tracts which are so characteristic in these animals, although it is not easy to understand how this happens. It is even more difficult to explain these degenerations otherwise, for they are fibres of the second ascending neurone series and their degeneration cannot be accounted for by destruction of the posterior root ganglia of the spinal cord unless such destruction has affected the cells of the second neurone which, from our present knowledge, is unlikely. It is certainly of interest that much more nerve degeneration is found in these animals between the mid-brain and the 6th cervical vertebra, and it is in these parts that the bone overgrowth is larger and the mechanical distorting effects on the central nervous system in this position correspondingly greater.

While, therefore, it is not possible as yet to state categorically that

bone overgrowth of the type described can be held responsible for the degree and distribution of nerve degeneration both in the central and peripheral nervous systems in these animals, the evidence in favour of this view is strong. In later publications the subject will be dealt with from the point of view of the cranial nerves and the overgrowth of the bone surrounding these nerves, and here again it will be seen that the evidence favours the view that degeneration of these nerves is related directly to the bone change and indirectly also to the increased intracranial pressure resulting therefrom.

There is one aspect of this problem which has not been dealt with here, namely the effect of the bone overgrowth on the blood supply to the brain and spinal cord. This may be of some significance because it is undoubted that the bony channels through which the vascular supply of the nervous system is carried are often much constricted in these -A animals as compared with normal animals. How far the displacement and constriction of the blood vessels interfere with the nourishment of the nervous tissue has not been sufficiently worked out. The foramina and other places where the blood vessels pass through bones, although narrowed, do not appear to be completely closed in any case so far examined, and it may be that the blood supply remains adequate. The point requires further examination.

IV. HOW DOES VITAMIN A AFFECT BONE GROWTH?

The gross effects of vitamin A deficiency on bone structure have been described and the question now to be considered is how these changes are produced, for with such knowledge it may be possible to determine the part played by vitamin A in normal bone growth.

A. *Histological examination*

In order to compare the histological appearance of bone tissue in +A and -A dogs, it is desirable to examine sections through identical parts of the same bones of litter mates. As previously mentioned, however, the complicated overgrowths of temporal and certain other bones make it difficult to obtain such sections. For this reason simple bones, i.e. femur shafts, have been used for comparison in this paper, although the changes are not so great as in the case of many other bones.

In comparing the cross-sections of the femur shafts of these +A and -A dogs, the most obvious differences are the thickening of the bone (see Pl. IV, figs. 8a and 8b) and the reduction in size of the marrow cavity in the -A dogs. In some cases the bones are also actually larger

in diameter, but this enlargement is not so common as the thickening of the bony layers. Whereas in the +A dogs the bones are comparatively compact, in the -A dogs they may be more or less compact towards the outer surface, but the greater part of the bone is cancellous in nature; the amount of this tissue depends on the extent of the deficiency and the length of the dieting period. The trabeculae of the cancellous bone generally run parallel with the outer surface.

If we now examine in more detail the sections represented in Pl. IV, figs. 8*a* and 8*b*, we see that in the normal dog the red marrow is found in a well-defined layer adjacent to the endosteal surface of the bone but clearly differentiated from it; there is no such marrow in the cavities of the bony layer. In the section from the -A dog, however, red bone marrow is diffuse and mixed up with the trabeculae in the inner third of the bony layer, while the outer two-thirds are free from it. If one of the trabeculae surrounded by red marrow is examined under a higher power, it will be seen that it is covered by a layer of active osteoblasts on the side nearer the marrow cavity, while on the outer side osteoblasts are reduced in number and not as active. The osteoclasts, however, are found mainly on the outer side of the trabeculae and judged from the size of the Howship's lacunae are more active when in that position. There are many more osteoclasts in the cancellous bone of the femur shafts of -A dogs than in the more compact bone of +A animals; for instance, in comparable sections of litter mates 380 osteoclasts were counted in a -A dog and only 144 in the corresponding +A animal. In these counts only cells adjacent to the bone were included, but it is interesting to note that the megakaryocytes were also increased in the -A animal.

Thus, while the more active osteoblasts on the endosteal side of the trabeculae lay down new bone, the osteoclasts on the outer side remove much of it. This probably accounts for the encroachment of cancellous bone on the marrow cavity in the -A animal and it seems from the material at present available to be the process by which the bone is converted from the compact to the cancellous state.

In addition to this effect of vitamin A deficiency on the osteoblasts and osteoclasts which are found in or near the red bone marrow, a similar effect can be seen on these cells in the subperiosteal region of bone. In this case also there is an increase in the activity of osteoblasts and probably also of osteoclasts, but the increase is not nearly so great as in the medullary bone. It follows, therefore, that the subperiosteal bone of -A dogs, although not so compact as in the normal animal, is much more compact than in the medullary two-thirds of the same bone.

The actual bone formed in the vitamin A deficient animal is probably not far removed from the normal bone and there is no increase of osteoid tissue. It is the gross arrangement of the bone which is so strikingly changed. It is probable, therefore, that the main effect of vitamin A on bone growth is to control the activity and number of osteoblasts and osteoclasts, primarily those associated with the bone marrow but also to a less degree the same cells in the subperiosteal region. In its absence these cells become more active, but the change seems to be one only in intensity and not in function, and the relative increase in these activities remains balanced, so that the total amount of calcified tissue does not greatly differ in the vitamin A deficient animal from that in one receiving this vitamin in abundance.

Although the effect of vitamin A deficiency on bone growth in these animals undoubtedly has a deeper significance than that described, the apparent mechanism seems to explain the bone changes.

B. *Chemical examination*

It has already been stated that the bone developed in vitamin A deficiency tends to be cancellous in those places where, with abundant vitamin A in the diet, it would have been compact. It is interesting to find on chemical analysis that the actual amount of calcium in corresponding bones of +A and -A animals is roughly the same. Taking an average amount of calcium in the femur shaft per 1000 g. of body weight in two groups of ten +A and ten -A animals, it was found that the +A group had 0.131 g., while the corresponding figure for the -A group was 0.134 g. In other words, under these experimental conditions, the thick cancellous bone of the -A animal contains little or no more calcium than the thinner more compact bone of the +A animal.

A second point of interest shown by chemical analysis is the relatively high fat content of the bones of some of these -A dogs as compared with that of similar bones of +A litter mates. This difference is particularly great in animals which have been sufficiently long on the diets to allow large bone changes in the -A dogs.

C. *The controlling effect of vitamin A on osteoblasts and osteoclasts*

It might be thought that the well-established fact that vitamin D influences bone formation would offer some kind of analogy as a guide in studying the vitamin A action on bony tissue. This is, however, not the case. The main function of the antirachitic vitamin is to promote calcification of bone: in its absence there is an excess of osteoid tissue, and the

mechanism of bone formation breaks down because this tissue remains largely uncalcified or is only slowly calcified. In other words, vitamin D stimulates a process—that of deposition in the matrix of the calcium salts essential for normal bone formation. Vitamin A, however, appears to influence the cellular elements involved in bone growth. Histological examination of the bone indicates that, unlike the stimulant calcifying action of vitamin D, the action of vitamin A is to limit and control. It has been described above how, in the absence of vitamin A, the activity of such cells as osteoblasts and osteoclasts becomes excessive.

This action of vitamin A deficiency on bone cells and structure must call to mind the earlier established action of vitamin A deficiency in epithelial hyperplasia. It is undoubted that a deficiency of this vitamin in some young animals causes characteristic changes in epithelium, the most common being hyperplasia of squamous epithelium, keratinization changes in such epithelium and, in some cases, metaplasia of columnar to squamous epithelium. Probably the fundamental change is overgrowth of epithelial cells of all kinds, keratinization and metaplasia being secondary to this overgrowth. These changes are more prominent in young rats than in other experimental animals and it is probable that this is the reason why it is widely accepted as the main pathological change produced by absence of vitamin A, since most nutritional research has been done on rats. Another reason why so much attention has been given to this phenomenon is that, in the rat at least, the epithelial changes are commonly associated with local infection, so that young rats nearly always develop multiple foci of infection when deprived of vitamin A. Other young animals such as rabbits and dogs, although not escaping these changes, do not seem to develop them to the same extent as rats. In rabbits and dogs stratified epithelium certainly becomes hyperplastic, but metaplasia is much rarer than in rats.

In the present work attention is drawn to another type of tissue which undergoes what may be the same fundamental change when vitamin A is deficient, namely excessive formation of osteoblasts and osteoclasts. It would appear then that the vitamin A not only controls the activity of epithelial cells but also of certain cells of mesoblastic origin. If this is established, it is obviously of some significance to cellular physiology in the mammal that there is a substance normally present in the body but not synthesized there, and provided of necessity in the food, whose main function is to control the number and degree of activity of certain epiblastic and mesoblastic cells. In its absence these cells increase abnormally in number and activity but do not, as do

ancer cells, change their function or act in a way different from the normal cells of the same type.

SUMMARY

1. A function of vitamin A is to influence the structure of growing bones, probably by limiting the number and the degree of activity of osteoblasts and osteoclasts. In its absence from the growing dog osteoblastic and osteoclastic activity is increased, thus resulting in proliferation of cancellous at the expense of compact bone and causing many bones to lose their normally fine moulding and outline and to become thickened and enlarged.

2. Some of the main positions of bone overgrowth in the skull and vertebral column produced by the vitamin A deficient diets used in these experiments are described. These overgrowths are related to degenerative changes in the brain and in cranial and peripheral nerves, of which accounts have been given in earlier publications.

3. Overgrowth of the cranial bones may press on and produce deformity of parts of the brain. The greatest hypertrophy is found in the bones forming the posterior fossa of the skull, so that the medulla oblongata, pons, cerebellum and nerves in close association with these parts of the brain are more particularly affected. Most of the cranial nerves of young dogs are liable to be compressed and to suffer destructive changes if the A deficient diets are continued over long enough periods (about 4 to 8 months).

4. The posterior root ganglia and the anterior root nerves of the spinal cord may also be squeezed and the nerve fibres destroyed by overgrowth of the vertebral bones. The bone overgrowth and nerve degenerative changes are greatest in the cervical region of the cord.

5. In advanced cases on these vitamin A deficient diets there is evidence of a substantial increase in intracranial pressure. The pressure of the cerebrospinal fluid in the cisterna magna may be double that of animals receiving similar diets containing vitamin A. Associated with these changes the cisterna magna and the 4th ventricle are diminished in capacity, while a degree of internal hydrocephalus (expansion of the 3rd and lateral ventricles) indicates increased pressure, probably transmitted from the posterior fossa.

Note. Since this paper went to press, the author has seen a publication by L. A. Moore and J. F. Sykes (*Amer. J. Physiol.* [1940], 130, 684) in which are described experiments showing increased cerebrospinal fluid pressure in calves on a ration deficient in vitamin A.

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EXPLANATION OF PLATES I-VI

PLATE I

Figs. 2a and 2b. Mesial sections through foramen magnum of +A (a) and -A (b) dogs, to show reduced capacity of cisterna magna in -A dog (2b). Here again the intrusion of the cerebellum into the cisterna magna in 2b is seen.

PLATE II

Figs. 3a and 3b. Coronal sections through 4th ventricles and lateral recesses (foramina of Luschka) of +A (a) and -A (b) dogs. Note the reduction in size, especially in dorso-ventral diameter of the 4th ventricle (IV), the packed appearance of the choroid plexus and the reduced diameter of the foramina of Luschka (Lu) of the -A dog (3b) as compared with the +A dog (3a).

PLATE III

Figs. 4a and 4b. Coronal sections through the lateral (Lat.) and 3rd ventricles (III) of +A (a) and -A (b) dogs. Note the distension of these ventricles (internal hydrocephalus) in the -A dog (4b) as compared with the normal +A dog (4a).

PLATE IV

Fig. 5a. Atlas vertebra of dog whose diet contained vitamin A. Note delicacy of outline and large vertebral canal.

Fig. 5b. Atlas vertebra of dog whose diet was deficient in vitamin A. Note bulbous appearance of all protuberances. Reduction in size of vertebral canal, but overall size of vertebra little affected.

Figs. 8a and 8b. Cross-sections of femur shafts of +A (a) and -A (b) dogs. (a) Showing nearly normal structure and (b) showing thickened cancellous shaft and great osteoclastic activity.

PLATE V

Figs. 6a and 6b. Transverse sections through the 5th cervical vertebra and the spinal cords of +A (a) and -A (b) dogs. Note the reduction in size of the vertebral canal and the compression of the spinal root ganglion in the -A dog (6b) as compared with the +A dog (6a): also the alteration in shape of the dorsal surface of the intervertebral disc (Int. Disc.) in 6b.

PLATE VI

Fig. 7a. Posterior root ganglion (P.R.G.) of dog receiving diet containing vitamin A.

Fig. 7b. Posterior root ganglion (P.R.G.) of dog receiving diet deficient in vitamin A. Note compression of ganglion between body and lateral processes of vertebrae.

THE EFFECT OF ETHER ON THE RATE OF ABSORPTION OF NORMAL SALINE SOLUTION FROM THE SUBARACHNOID SPACE

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(Received 21 December 1940)

AN account has been given in an earlier paper [Bedford, 1939] of a series of observations on the effect of variations in carbon dioxide tension on the rate of absorption of normal saline solution from the subarachnoid space of dogs. The animals in these experiments were usually anaesthetized with ether although in some instances amytal was the anaesthetic. An attempt has been made in the following experiments to determine the effect on absorption (1) of the administration of ether to animals previously anaesthetized with certain basal anaesthetics, and (2) of variation in the rate of administration of ether to animals already under the influence of this anaesthetic.

EXPERIMENTAL PROCEDURE

The animals used in these experiments were dogs varying from 7 to 10 kg. in weight and from 2 to 4 years of age. In the first group of experiments the animals were first anaesthetized with one of the following anaesthetics: (1) amytal, (2) avertin, (3) chloralose. Amytal anaesthesia was obtained by introducing an aqueous solution of amytal (Lilly) into the peritoneum. The method of administering the amytal and the precautions to be observed have been described in detail in an earlier paper [Bedford, 1938]. Avertin anaesthesia was obtained by injecting avertin fluid (Bayer) in a 2.5% solution in water into the peritoneum. It was administered in a proportion of 0.3 c.c. solution per kg. body weight. Chloralose (B.D.H.) was administered intravenously in a 2.5% solution in water. About 6 c.c./kg. of this solution were required for complete anaesthesia. The solution, raised to body temperature, was introduced into a leg vein. This procedure presents little difficulty if care be taken to choose a quiet animal and to anaesthetize

painlessly the skin overlying the vein with a little procaine solution. Whether amytal, avertin or chloralose be used as basal anaesthetic it is important that anaesthesia should be obtained as a result of one single administration. After surgical anaesthesia had been attained, a tracheal tube was inserted and the latter connected with a pump which maintained respiration at its normal rate and amplitude. A simple device which permitted a regulated quantity of ether vapour to be added to the air from the pump was interposed between the animal and the pump.

In the second group of experiments the animals were anaesthetized in a closed box with ether. They were then removed from the box, a tracheal tube introduced and connected with the pump as in the first group of experiments. Although no method was discovered by which the effect of the administration of ether to unanaesthetized animals could be recorded, it was possible to make observations on the effect of variation in the quantity of ether administered to animals already under the influence of this anaesthetic. The rate of inflow of normal saline solution into the subarachnoid space was determined in precisely the same way as in earlier experiments [Bedford, 1938]. Careful control experiments were performed with each anaesthetic to determine whether it exerted any influence on absorption rate.

RESULTS

The effect of ether on the absorption of normal saline solution in animals under amytal anaesthesia

Control experiments were first performed to determine the effect of the administration of amytal in doses large enough to produce anaesthesia, on the absorption of normal saline solution from the subarachnoid space. The drug was found to bring about a progressive reduction in absorption which generally began about 1-1½ hr. after the administration of amytal. After 2 hr. inflow was frequently at half or even less of its original rate. These results confirm those obtained in an earlier series of experiments [Bedford, 1938].

When ether was administered to an animal under amytal anaesthesia, an immediate reduction occurred in the rate of inflow. A typical experiment is illustrated in Fig. 1. It will be seen that a great decrease in inflow occurs during the first 3 min.; fluid frequently flows from the animal towards the recording apparatus. This is succeeded by a period during which absorption takes place, although at a reduced rate. In the majority of experiments the reduction in absorption remains constant and persists as long as the ether is administered. The longest period of

observation was 33 min. In a few instances the rate of inflow after a period of 10–15 min. tends to approach that before the administration of the ether. An increase in the rate of the administration of the ether caused a diminution in the rate of inflow while a decrease had the opposite effect. If the quantity of ether administered was increased to such an extent that spontaneous respiration was abolished, the diminution of inflow was still maintained and persisted until the onset of cardiac failure. On replacing the ether by air, a sudden increase in inflow occurs

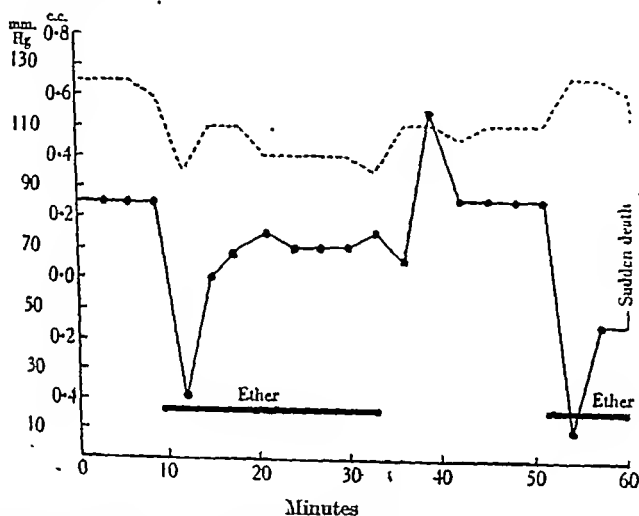


Fig. 1. The effect of ether on the absorption of normal saline solution in a dog anaesthetized with amytal. The ether was administered, at the end of the experiment, in a concentration which produced sudden death. The rate of inflow was determined at a constant pressure of 350 mm. normal saline solution throughout the experiment. It is expressed in terms of volume of solution entering the subarachnoid space during periods of 3 min. The systolic pressure in the femoral artery is recorded in mm. Hg. —•— Volume of normal saline solution absorbed. ---- Systolic pressure in femoral artery.

during the first 3 min.; the increase may be at twice or three times the rate which obtained before the administration of ether. This temporary increase soon declines and absorption proceeds at the initial rate. Occasionally, however, the sudden increase in inflow is delayed for several minutes after the administration of ether has ceased and inflow continues at its reduced rate. This phenomenon was not always associated with a rapid rise in blood pressure which sometimes followed the administration of air. The length of time over which the ether had been administered seemed to be without influence on these reactions. They were also affected to a relatively slight extent by the depth of amytal anaesthesia. Profound

amytal anaesthesia, however, tended to reduce their amplitude and duration. Fifteen experiments were performed with animals under amytal anaesthesia; six of these were controls.

*The effect of ether on the absorption of normal saline solution
in animals under avertin anaesthesia*

Control experiments were first performed to determine whether avertin anaesthesia has any effect on the rate of absorption of saline solution from the subarachnoid space. Surgical anaesthesia was obtained in a few minutes after intraperitoneal injection and persisted on the average for about an hour; recovery then took place, rapidly. It was rarely possible, therefore, to continue the experiments for much more than an hour.

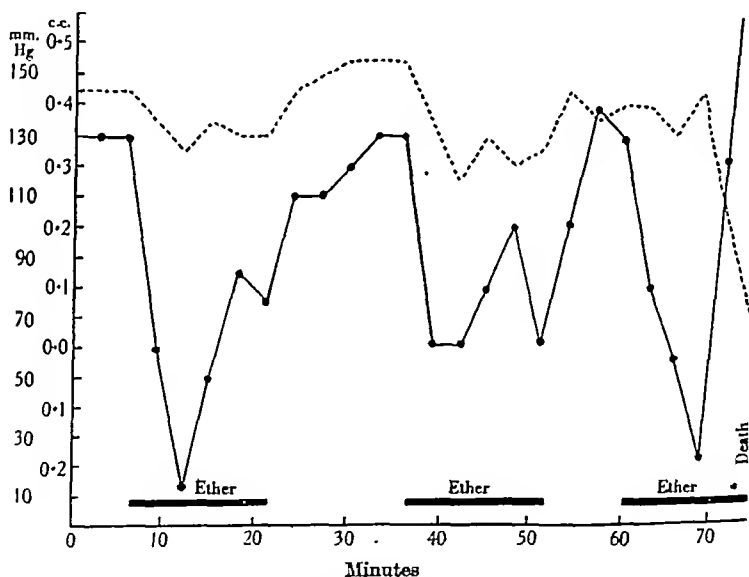


Fig. 2. The effect of ether on the absorption of normal saline solution in a dog anaesthetized with avertin. The concentration of ether was increased at the end of the experiment until death resulted from cardiac failure. The constants are the same as those in Fig. 1.

No significant variation in the rate of inflow was observed over this period.

The administration of ether to an animal anaesthetized with avertin produced effects almost identical with those observed in animals under amytal anaesthesia. A typical experiment is illustrated in Fig. 2. In this instance the animal was finally killed by increasing the concentration of ether until cardiac failure resulted. The cardiac failure was relatively

slow in onset and thus differed from the sudden failure which occurred in animals anaesthetized with amytal. The effects on inflow of the administration of ether after the influence of avertin had begun to wear off did not differ from those which accompany variations in the rate of administration of ether to animals already under the influence of this anaesthetic. Seven experiments were performed with avertin, three of these were controls.

The effect of ether on the absorption of normal saline solution in animals under the chloralose anaesthesia

Control experiments showed that chloralose, when administered in the manner indicated, brought about a reduction in the rate of inflow. This

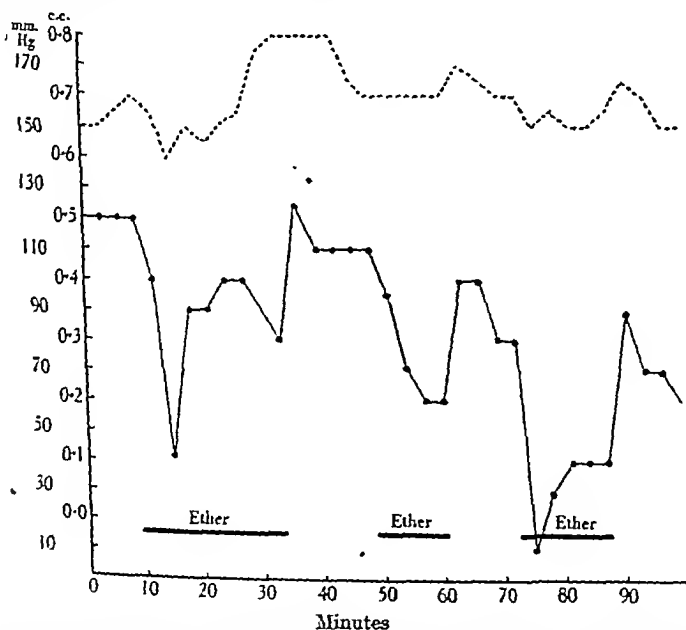


Fig. 3. The effect of ether on the absorption of normal saline solution in a dog anaesthetized with chloralose. The effect of the chloralose on absorption is also evident in this experiment. The constants are the same as those in Fig. 1.

reduction was gradual in onset and generally became apparent about 1 hr. after the administration of the drug; after 2 hr. inflow may be reduced to half its initial rate. The administration of ether to an animal under chloralose anaesthesia brought about a reduction in the rate of inflow which resembled that observed when amytal or avertin were used as basal anaesthetics although it was rarely so profound. A typical

experiment is illustrated in Fig. 3. The gradual reduction in inflow caused by chloralose is clearly evident. It should also be noted that the effect of ether on absorption is relatively greater towards the end of the experiment than at the beginning. A similar phenomenon was frequently observed with amytal and with avertin. Ten experiments were performed with chloralose; four of these were controls.

The effect of variations in the rate of administration of ether on the absorption of normal saline solution in animals already anaesthetized with ether

Experiments were first performed in which ether was administered so as to maintain a steady state of surgical anaesthesia. Under these circumstances inflow remained unchanged except for minor variations

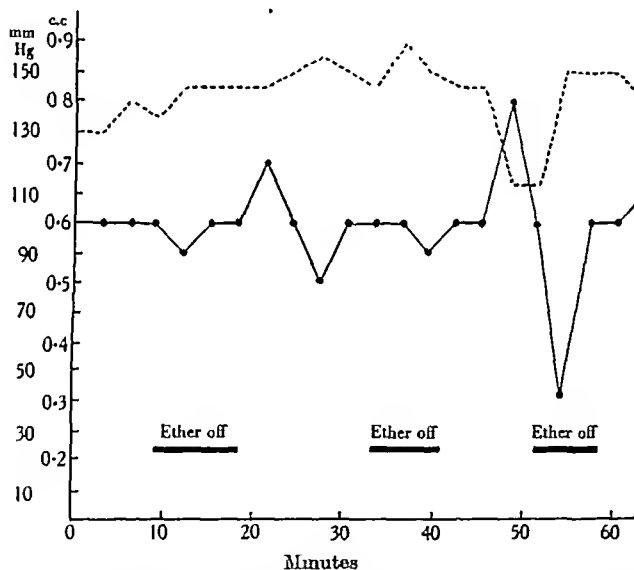


Fig. 4. The effect of variations in the rate of administration of ether on the absorption of normal saline solution in a dog already under the influence of this anaesthetic. The blood pressure was depressed temporarily towards the end of the experiment by increasing the concentration of ether. The constants are the same as in Fig. 1.

over periods of two or more hours. These findings are in agreement with those of Weed & Hughson [1921].

An attempt was next made to study the effect on absorption of discontinuing the administration of ether. An experiment of this type is illustrated in Fig. 4. It will be observed that no change occurs in the rate of inflow when ether is replaced by air. Similarly the addition of

ether is unaccompanied by any change in inflow. The blood pressure was purposely depressed by increasing the depth of anaesthesia towards the latter part of the experiment. It will be observed that an immediate rise in blood pressure occurs on stopping the ether. This is associated with a temporary reduction in inflow. Any change in rate of inflow observed in these experiments could always be accounted for on a basis of variation in blood pressure.

Ten experiments were performed on animals anaesthetized with ether; four of these were controls.

DISCUSSION

The effects of ether on the absorption of normal saline solution from the subarachnoid space resemble each other so closely whether amytal, avertin or chloralose be used as basal anaesthetic that it would seem justifiable to conclude that in each instance they are brought about in a similar manner. In an earlier series of experiments [Bedford, 1939] carbon dioxide in a concentration of 5-10 % by volume in air was found to have effects very similar to those of ether on animals under amytal anaesthesia; ether is relatively more powerful in its action. It was concluded for reasons that have already been advanced that carbon dioxide produces its effect on absorption mainly as a result of its action on the cerebral blood vessels. We are accordingly led to conclude that ether also brings about its effects in a similar way. No absolute measurements were made of the concentration of ether administered. The concentrations of ether which can safely be administered to an animal already anaesthetized with basal anaesthetic must be low. Yet these low concentrations had a more powerful and prolonged action on absorption than carbon dioxide in concentrations of 10 % by volume in air. It would appear therefore that ether is a more powerful dilator of the cerebral blood vessels than carbon dioxide.

Although a constant pressure of 300-400 mm. normal saline solution was maintained in the subarachnoid space throughout these experiments, a very different state of affairs would obtain in the intact animal. The sudden increase in volume of the brain caused by vascular dilatation would bring about a great rise in the pressure of the cerebrospinal fluid; this would be accompanied by an accelerated rate of absorption [Weed, 1935; Bedford, 1938]. It is conceivable that the cerebrospinal fluid may be completely absorbed in some regions and the subarachnoid channels obliterated. In addition to local obliteration of the subarachnoid channels, the increase in brain volume may be accompanied by distortions and displacements of varying magnitude.

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The effect of variations in the rate of administration of ether on the absorption of normal saline solution in animals already anaesthetized with ether

Experiments were first performed in which ether was administered so as to maintain a steady state of surgical anaesthesia. Under these circumstances inflow remained unchanged except for minor variations

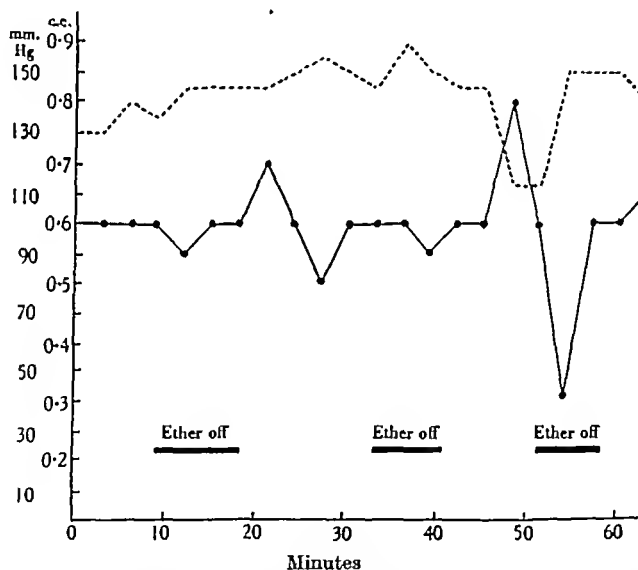


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For humane reasons the experiments on the effect of variations in the rate of administration of ether to animals already under the influence of this anaesthetic are of necessity incomplete. Nevertheless, no dramatic change was observed in absorption rate such as occurred when ether was administered to animals anaesthetized with one of the three basal anaesthetics. Whenever a change did occur, it could be accounted for in terms of variation in systemic blood pressure. It was rarely possible to discontinue the administration of ether for longer than 6-9 min. At the end of this period the animals were still in a state of light anaesthesia and the degree of ether saturation must have been relatively high. The animals were therefore in a very different condition from those anaesthetized with basal anaesthetic in which ether saturation was at all times low.

It would appear therefore that ether brings about an intense degree of dilatation of the cerebral vessels at a period when the tissue saturation is low. In ordinary ether anaesthesia this phase is rapidly passed over and the cerebral vessels recover to a great extent their original tone. It has been shown, for example [Bedford, 1939] that the administration of carbon dioxide to animals anaesthetized with ether brings about changes in the rate of absorption of normal saline solution from the subarchnoid space which closely resemble those produced by carbon dioxide or ether on animals under amytal anaesthesia, although they are of less intensity. Moreover, the average rate of absorption in animals anaesthetized with ether and at the beginning of amytal anaesthesia is the same. It is suggested that a degree of ether saturation at which the cerebral vessels cease to be dilated cannot be attained in animals anaesthetized with any of the three basal anaesthetics under consideration even when ether is administered until respiratory and ultimately cardiac failure results.

SUMMARY

1. The administration of ether to animals anaesthetized with amytal, avertin or chloralose caused a marked reduction in the rate of absorption of normal saline solution from the subarachnoid space. This generally persisted as long as ether was administered. Replacement of ether by air caused a sudden increase in inflow; this subsided after 3-9 min. when absorption proceeded at its normal rate.

2. Variation in the rate of administration of ether to animals already under the influence of this anaesthetic was unaccompanied by any significant change in the rate of absorption.

3. It is concluded that the variations in rate of absorption encountered in these experiments can be adequately explained in terms

of change in calibre of the cerebral blood vessels. Ether dilates the cerebral vessels when the degree of ether saturation is low; this action passes off as saturation increases. A degree of ether saturation at which the cerebral vessels cease to be dilated cannot be attained in animals anaesthetized with amytal, avertin or chloralose.

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THE INFLUENCE OF A TROPICAL ENVIRONMENT UPON THE BASAL METABOLISM, PULSE RATE AND BLOOD PRESSURE IN EUROPEANS

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Evidence which has been accumulated on this subject indicates that depression in the B.M.R. is a common reaction in those going to a tropical climate from temperate zones [Almeida, 1920; Knipping, 1923; Edmarsh, 1926; Sundstroem, 1926; Earle, 1928; Teding van Berkhout, 1930; Radsma, 1931; Radsma & Streef, 1932; Miller & Benedict, 1937, others].

Some of these observers have gone further into the question by dividing their subjects into categories based upon the length of time of tropical residence. Knipping [1923] found the B.M.R. showed a greater depression in a subject who had lived in the tropics for 10 years than in one who had been resident for a shorter period. His observations appear to have been made, however, on only four subjects.

The question has been investigated much more extensively by Radsma [1931] and Radsma & Streef [1932]. Radsma's results, summarized in Table 1, gave averages of -7.9% (Benedict standard) for twenty-eight subjects of less than 3 months' residence in the tropics, and -6.8% for twenty subjects of 1 year or more of residence. To this he adds an interesting series of subjects of European origin, 17-22 years of age, born and bred in the tropics. The average for this group is only -1.7% from the Harris-Benedict standard, a value which would be accepted as normal for a temperate climate. The difference of this average from the normal was statistically significant. There is no evidence from these last results that the depression of the B.M.R., which undoubtedly appears to be present in groups of Caucasians in the tropics, increases with prolonged residence; nor do the results confirm the suggestion made by Ocampo,

Cordero & Concepcion [1930] that the extremely low values found by Almeida might be due to his subjects being born and bred in the tropics though of Caucasian origin, whereas the groups examined elsewhere had only been resident for a relatively short period of time in a hot climate. It has always been difficult to obtain subjects of pure European descent who live in the tropics continuously, as far as the American, British and Dutch tropical colonial possessions are concerned.

The effect of short spells of warm climate has been investigated, with varied results, by Caspari & Schilling [1886], Eijkmann [1898], Young [1920], and Martin [1930].

Rasma & Streef [1932] determined the B.M.R. of nine subjects within 3 months of their arrival in the tropics, and again on the same subjects after 2 years' residence. In no individual case was there a greater depression of the B.M.R. after 2 years than that found during the first 3 months. In several cases the two values were almost the same, and all values were definitely below those given as standards for temperate climates. These results indicate that, whatever the reaction of the individual to an increase in environmental temperature (and a fall in B.M.R. appears to be the commoner type of variation), the change does not progress with time after the first year to any marked extent in the majority of individuals.

Concerning changes in the pulse rate, observations made by a number of physicians working in tropical climates have led to the general impression that the rate increases at first but decreases with residence, and acclimatization is a factor which has been held to be responsible for this. The systolic and diastolic blood pressures are generally regarded as being lower in tropical circumstances than in temperate, although it is not wise to accept as definite evidence averages found by different observers where the method of measurement is not standardized to a greater degree.

Musgrave & Sison [1910] measured the blood pressure of Caucasians in Manila. They found that the average systolic blood pressure decreased with length of residence. In those who had spent less than a year in the tropics this value was 124 mm. Hg. In another group with from 5 to 10 years of residence, the average was 116 mm. Hg, and in a group with over 10 years' residence, 113 mm. Hg. The number in these groups was not very large. Chamberlain [1911], from observations upon American soldiers in Manila, concluded that the pulse rates were, on the average, a little above the standard in America and that the blood pressure average showed no tendency to fall with residence. The average systolic

pressure was 115 mm. Hg and this was within the range assumed to be normal for Caucasians of that age in a temperate climate. Larger groups were used than those of Musgrave & Sison.

Roddis & Cooper [1926], from observations on naval officers in the tropics, suggested that a fall in systolic and diastolic pressures took place on account of the climate. Their averages were 10 mm. lower than those given in standard text-books of medicine in America. Knipping [1923] found a very definite lowering of the systolic blood pressure in every one of fourteen subjects examined during a voyage from Hamburg to Java. Radsma and his collaborators [1936] measured the pulse rate and blood pressure in subjects travelling between a temperate and tropical zone. On the average the pulse frequency was slightly higher and the blood pressure slightly lower in the tropics. The average blood pressure measured in a group in Amsterdam showed a difference from that of a group measured in Batavia which was not significant.

The present paper records observations of both B.M.R. and blood pressure carried out upon Europeans resident for varying periods in Singapore.

METHODS

Personnel. The subjects were soldiers living in barracks and were divisible into two groups:

Group A. Thirty-five subjects whose residence in the tropics did not exceed 6 months. Certain of these were examined after varying periods of residence in the following way:

- (1) Nine subjects at the end of 2 months' residence.
- (2) The same subjects at the end of 8 months' residence.
- (3) Sixteen subjects at the end of 6 months' residence.
- (4) The same subjects at the end of 1 year's residence.

Group B. Thirty-five subjects at the end of 2½ years' residence.

The groups were closely comparable with each other in respect of diet, exercise, and occupation, and similar in physical data, as shown in Table I. All the subjects were in a good state of health. The data of Table I indicate that the group which had been resident in the tropics for 2½ years was in no way less fit than the group made up of individuals who had been less than 6 months in the tropics. It should be pointed out, however, that group B had been under army training for over 2 years, while a certain percentage of group A had only recently joined the unit from civilian life.

Investigations were undertaken upon single individuals month by month as well as the examination of groups. Twelve soldiers investigated

in this way had to leave the colony on duty after only a few months' observation and measurements at intervals were continued upon two civilian subjects only. Both of these subjects were physically fit and, although engaged upon what is classed as a sedentary occupation during the day, took regular exercise in the evenings. The regular examination of these subjects was continued at intervals of 2-3 months throughout 2 years in the tropics.

Technique. All subjects were conveyed to the laboratory by car after a night's rest and without food. Precautions were taken to prevent their indulging in any form of exercise on getting up, and on arrival at the laboratory, they lay on camp beds set well apart from each other and dozed or slept during the preliminary period. Strict supervision was kept to ensure complete rest and quiet, the blinds being partially lowered and sources of sound muffled.

Two basal metabolism measurements were made upon each subject on four separate days. The temperature, pulse rate, and blood pressure were also measured upon the four separate days, with the subject in the basal and post-absorptive state. Of the two daily metabolism measurements, one was made between the first half-hour and hour, and the other during the second hour. A third measurement was made if either was unsatisfactory. The pulse rate and blood pressure were taken at the conclusion of the first metabolism experiment and the mouth temperature before the second. Blood pressures were measured by the auscultatory method. The procedure was repeated exactly on four separate, usually consecutive, days, making a total of eight metabolism experiments and four basal pulse, blood pressure, and temperature observations in each individual.

The B.M.R. was determined with a Benedict recording spirometer. Two spirometers were used throughout and a third was also in use on certain occasions. All three were subjected to alcohol check experiments, the technique of Barrett & Robertson [1937] being followed closely. A half-face mask was used in preference to mouthpiece and noseclip, as it is more comfortable. In every experiment facepiece and valves were tested for leaks, and the usual test of weighting the spirometer bell was carried out. Precautions were taken to ensure that the temperature throughout the apparatus did not exceed that of the surrounding air by more than 2° C. at the beginning of each experiment. This sometimes involved removal and spreading out of the 'Calsoda' between experiments, since at the tropical room temperature this tended to become excessively warm during the course of several experiments. The 'Calsoda'

was renewed after every twenty experiments, which allowed a very wide margin of safety.

Some forty control experiments were performed, using the Douglas-Haldane in addition to the spirometer technique. When performed, the immediately preceded the second spirometer determination. The results omitted for lack of space, showed good agreement between the two methods.

RESULTS

The figures given at the foot of Table I show that no significant difference exists between the average B.M.R. determined for thirty-five subjects during their first 6 months in tropical residence (some estimate in the second and some in the sixth month of residence) making up group A, and that found for thirty-five subjects of a closely similar category who had completed $2\frac{1}{2}$ years' residence forming group B. The values found for both groups are 5-6% below the standard value for a temperate climate, but, allowing for the probability that this depression of metabolism has occurred largely during the first 6 months of residence, the average values during the next 2 years appear from these group averages to remain relatively constant.

An examination of the same groups of individuals on more than one occasion during residence in the tropics was made upon nine of the above subjects at the end of 2 months and later after 8 months in the tropics and also upon sixteen of the subjects at the end of 4 months and later at the end of 1 year in the tropics. The values found in these groups are shown in Table II. The average B.M.R. falls progressively (from a value which probably approximates to the standard for a temperate climate on arrival) in the course of the first year of residence. Statistical analysis shows this change to be significant. No such variation is apparent for the pulse or systolic and diastolic blood pressure. Although these average values show a fall, it was clear from the results of the individual cases that there is a marked difference between individuals in their reaction to a tropical environment and whilst fifteen of these subjects exhibited a clear depression of the B.M.R. ten of them showed little or no change whatsoever on reinvestigation after a period of 6 months.

The figures shown in Table III illustrate the effect of a tropical climate during the course of 2 years upon two civilian subjects in whom the B.M.R. is definitely affected. The pulse rates of these subjects show an initial fall but do not at all times show a close correlation with the values of the B.M.R.

METABOLISM OF EUROPEANS IN TROPICS

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TABLE I. Comparison of physical and physiological values found for Europeans
(Two groups A and B consisting of thirty-five subjects in each)

	Group A	Group B
Age	22	23
Weight, kg.	64.2	64.4
Standard deviation	6.5	5.1
Standard error	1.1	0.9
Height, cm.	171.2	172.1
Standard deviation	7.4	4.7
Standard error	1.3	0.8
Surface area, sq.m.	1.74	1.75
Standard deviation	0.3	0.2
Standard error	0	0.05
Vital capacity, c.c.	4337	4565
Vital capacity (based upon surface area, % normal standard)	98	103
Blood haemoglobin content	120	127
Haldane standard	96	101
Sahli standard	16.5	17.5
g. %		
*Cardiac efficiency	98.9	99.7
% of normal		
Dynamometer (grip pressure, lbs.)	115	124
Right hand	111	119
Left hand	97.8	97.8
Mouth temperature ° F.	58.8	55.2
Pulse rate per min.	6.7	5.8
Standard deviation	1.1	1.0
Standard error	102.8	100.3
Systolic blood pressure	8.6	8.2
Standard deviation	1.1	1.1
Standard error	61.3	61.1
Diastolic blood pressure	6.2	6.5
Standard deviation	1.0	1.1
Standard error	41.5	39.2
Pulse pressure		
Basal metabolism		
Oxygen consumption in c.c. per min.	228	225
Calories per sq. m. per hr.	37.88	37.04
Calories per 24 hr.	1583	1563
Percentage deviation from the Harris-Benedict standard	-4.3	-5.7
Percentage deviation from the Aub-DuBois standard	-4.5	-6.1

The basal values given are the mean values of third and fourth day estimations of measurements performed upon four successive days.

* Test applied according to Master & Oppenheimer [1929].

TABLE II. Mean values from nine group A subjects examined after 2 and 8 months
and sixteen group A subjects examined after 4 and 12 months

Residence, months	2	4	8	12
Weight, kg.	63.2	66.1	63.7	65.1
B.M.B. difference, DuBois	-1.9	-6.2	-3.8	-10.0
Pulse rate	54	57	52	57
B.P. systolic	103	103	100	102
B.P. diastolic	62	62	60	62
Pulse pressure	41	41	40	40
Haemoglobin, g. %	16.5	17.0	16.7	16.6
Total white cells, per c.mm.	5668	5434	5411	5576
Polymorphs, %	67.4	62.6	60.1	60.7
Lymphocytes, %	18.3	20.4	22.5	22.4

TABLE III. Values found in European subjects investigated at intervals during tropical residence

Subject	Date	Months of tropical residence	Weight, kg.	H.M.H. difference, DuBois	Pulse rate	Systolic pressure	Diastolic pressure	Pulse pressure	Hæmo- globin, g.	Red cell count, per c.mm.	White cell count, per c.mm.	Polymorph, %	Lymphocyte, %	Vital capacity, c.c.
G. E. B.	Nov. 1938	4	71.4	-3.2	49	105	66	39	16.2	4.945	6957	87.2	5.6	3900
"	Mar. 1939	7	70.5	-10.3	49	97	63	34	16.5	4.905	5206	66.66	15.9	4150
"	June 1939	11	68.6	-11.3	43	93	55	38	15.0	4.230	4522	64.9	21.6	4600
"	Aug. 1939	13	68.6	-18.2	54	92	62	30	15.3	4.530	5744	65.2	20.1	4400
"	Nov. 1939	16	69.5	-10.5	50	92	56	36	15.7	4.635	6955	68.9	17.9	4590
"	April 1940	21	71.8	-14.2	55	99	62	37	15.5	4.565	6722	66.8	17.3	4050
"	July 1940	24	72.3	-13.3	56	91	63	28	15.7	4.800	4756	59.3	21.3	4309
"	Oct. 1940	27	72.3	-15.9	52	93	61	32	15.7	4.640	5720	58.3	28.1	4650
D. A. F.	Nov. 1938	4	71.8	-5.2	65	113	76	37	17.3	5.670	5200	73.7	10.8	4550
"	Mar. 1939	7	71.4	-7.0	63	106	73	33	15.1	5.620	4900	66.5	19.3	4400
"	June 1939	11	70.0	-13.8	59	103	67	36	15.8	4.980	4604	60.2	22.1	4400
"	Aug. 1939	13	71.4	-12.5	58	104	68	36	15.1	4.855	5188	72.6	15.6	4850
"	Nov. 1939	16	72.7	-10.3	60	105	72	33	16.2	5.000	4500	66.1	18.2	4700
"	April 1940	21	72.7	-12.0	62	106	72	34	15.5	5.170	5844	74.5	9.5	4800
"	July 1940	24	71.8	-18.5	58	109	73	36	15.0	5.099	4100	65.8	14.6	4750
"	Oct. 1940	27	73.6	-17.4	59	104	71	33	16.1	5.200	5830	59.9	19.7	4600

The date given represents the first of the three consecutive days' investigation.
 The basal values given are the mean of the three consecutive days' investigation.

TABLE IV. B.M.R. of five normal subjects (Chinese students) during different seasons of the year in Singapore

Reference no.	Age	19 October 1938		11 January 1939		5 July 1939	
		Surface area	Difference DuBois	Surface area	Difference DuBois	Surface area	Difference DuBois
208	21	1.55	-17.2	1.55	-16.3	1.60	-18.0
212	18	1.61	-10.1	1.61	-11.4	1.62	-11.9
219	20	1.65	-13.3	1.65	-7.2	1.64	-11.9
225	23	1.39	-4.1	1.39	-7.2	1.41	-5.5
228	20	1.65	-7.1	1.65	-7.1	1.66	-3.3
Mean	20	1.57	-10.4	1.57	-9.8	1.59	-10.1

Each value given in the tables was determined, as has been stated, from the results of six to eight experiments performed on 3-4 successive days. All those examined, even in the larger groups, were therefore well accustomed to co-operating and can be regarded as trained subjects. The remote possibility that variations might be due to a climatic influence of a seasonal nature was excluded by investigating at intervals in the course of the year a group of five Chinese medical students born in Malaya. These subjects exhibited no sign of a seasonal fluctuation (Table IV).

DISCUSSION

The average B.M.R. found in this investigation on groups of Europeans living in a tropical environment is lower than would be expected in a temperate climate and this confirms the results of other observers. The average values found in two separate groups of normal individuals suggest also that this depression in the B.M.R., which takes place during the early months of residence, probably remains relatively constant thereafter, since the type of subject making up both groups is shown to be closely comparable.

A further examination of a number of individuals belonging to the group investigated shortly after arrival shows, on the average, that a definite fall in B.M.R. is taking place in the course of the first year. This examination also demonstrates that normal subjects can be differentiated in two distinct types, namely (a) those who react to a tropical environment by a definite lowering of the B.M.R. and (b) those in whom no effect on the B.M.R. is shown during this time of residence in the tropics. Although all subjects may be chosen on the grounds of showing complete physical fitness, the depression of the average value in such groups indicates that the second type usually tends to predominate.

Individuals grouped according to these two distinct categories have been investigated with a view to determining whether any further physical or physiological factors exist which are in any way peculiar to

either type, such as might be found in the physique, fairness of the skin, health (loss of appetite or sleep) or in the initial value of B.M.R., pulse or blood pressure or nervous or endocrine make-up of the individual as indicated by the physical appearance, polymorph-lymphocyte ratio or reactions to exercise tolerance tests. No suggestion has so far been found of any significance which might indicate such types but the numbers investigated up to the present are not sufficient for conclusions to be drawn in this respect. Whatever category they might belong to, all the subjects investigated were as closely comparable as possible on the basis of diet, exercise and environment since they were under training and in barracks.

An examination of results obtained on two subjects in civil life, who belonged to the type which exhibited a fall in B.M.R. in response to tropical environment and who exhibited a good standard of physical fitness, shows that the depression of the B.M.R. is maintained fairly constantly throughout the course of over 2 years of tropical residence. It is of interest also to note that these two subjects underwent a period of military training in July, August and September 1940 and that no marked variation in the B.M.R. resulted in either case.

The nature of the reaction which appears to affect these subjects may be a direct response to change in temperature similar to that described by Young [1920] and Martin [1930], where a sudden variation in B.M.R. (a marked fall in the case of Martin) was found to take place with a sudden extreme rise in temperature. In both these cases the investigators themselves acted as the subject throughout a heat spell. On the other hand, in view of the time taken for the B.M.R. to reach its final level of depression in the subjects in this present investigation, it would appear that the reaction in this case is probably due to a more gradual internal change resulting only indirectly from the altered environment.

The environmental changes to which an individual would be subject coming from England to Singapore may be summarized briefly as follows:

(a) A higher external temperature with an average of 83° F. during the day and 76° F. at night, which would remain relatively constant throughout the year, changes of more than 10° F. being exceptional.

(b) A barometric pressure of 760 mm. Hg which remains relatively constant from day to day throughout the year with diurnal variations.

(c) A moisture content of the atmosphere about three times that of England with a relative humidity which shows variations throughout the day depending upon sunshine, prevailing wind, temperature and pressure changes, with a greater variation than is general in England.

(d) A variation in prevailing winds which is roughly demarcated by the opening and close of the rainy season and gives rise to a greater number of hours of rainfall and increased total humidity at certain times of the year.

(e) More intense sunshine throughout the year which differs from that found in temperate climates in quality as well as in intensity.

(f) Electrical variations in the atmosphere which are not present to the same degree or frequency in temperate climates.

The high temperature which is experienced from the time of arrival throughout the years of residence is probably primarily responsible for the physiological reactions noticed. A high external temperature is a constant factor in all investigations of this type made upon groups of normal Caucasians in different localities where the average B.M.R. has been found to be depressed. In Malaya this lowering of the B.M.R. in European groups is similar to that found in the groups of the Northern Indians coming from the Punjab but not equivalent to the lowered B.M.R. found in groups of Chinese, Malays and Southern Indians [MacGregor & Loh, 1940].

A high degree of humidity will probably contribute to the extent that it renders the body less capable of responding to the increase in temperature by increased rate of evaporation of moisture. The extent to which the humidity of the atmosphere is responsible for the variations found would be indicated by comparison with investigations made in a climate where the temperature was the same but the air was relatively dry, such as is found in certain subtropical zones.

The barometric pressure cannot be considered as a factor likely to have contributed to the changes in question.

There is no suggestion that those who spend more time in the sun, as indicated by occupation or degree of redness of the skin, have an average B.M.R. which differs from those who do not, and who show a pallid or sallow complexion, but so many other factors are involved in such cases that it would be difficult to estimate the extent of this effect were it present. Although the sunshine is abundant in Malaya it does not contain such a high percentage of rays at the violet end of the spectrum as may be found in many other places with less sunshine. This relative diminution in ultra-violet, in spite of the intensity of the infra-red, is presumably due to the large amount of water vapour usually present in the air, and accounts to some extent for the infrequency with which true tanning takes place in those exposed to the sun, most of whom show an intense red reaction which is followed by peeling of the skin and leaves a

comparative pallid or sallow complexion unless maintained by regular exposure. It also accounts, in all probability, for the relative absence of sunstroke, the worst effects of exposure being largely confined to burns upon the skin in most cases. Since it is the ultra-violet rays which are likely to bring about any variation in physiological activity which might be expected as the result of increased sunlight, a high degree of variation due to this factor is hardly probable.

The electrical changes in a tropical climate like Singapore show a marked degree of difference both in intensity and frequency from what is usually experienced in a temperate climate, and those changes are often accompanied by thunderstorms. Such altered conditions in atmospheric ionization might contribute to the changes described.

An individual coming to the tropics from a temperate climate modifies his diet and habits. Alterations in diet, however, are not so great at the present time as they were some years ago. It was often asserted by the older writers on tropical conditions, and supported by the experience of a large number of individuals, that there was a very definite decrease in the calorie intake and that this was the result of diminished exercise and decrease in appetite and a cause of lowered metabolism. It would not be possible for a general statement to be made on this question, since the modifications which the diet and habits of an individual will undergo depend not only upon the individual himself but upon the part of the tropics in which he lives. In Singapore, where the observations under discussion have been made, the facilities for transport and cold storage are such as to allow a wide choice of foods to suit the diet and palate, and consequently the average person coming there is unlikely to have to alter the type of foodstuffs which make up his usual diet to any appreciable extent. Nor is the calorie intake likely to be decreased in the healthy adult.

The rations of the subjects investigated by us had the following composition: protein, 129 g.; fat, 157 g.; carbohydrate, 479 g.; calories, 3958; these values cannot be considered in default of those in a temperate climate.

The average weight of those subjects who exhibited a marked variation in B.M.R. after 6 months' interval did not alter nor did the two subjects examined over 2 years exhibit a loss in weight.

It is probably safe to assume that the average individual takes more exercise in Malaya at the present time than he would be having in England throughout the year. This may account for the appetite being maintained and sometimes increased.

The values for the B.M.R., pulse rate and blood pressure are normally kept relatively constant from month to month largely through the agency of the nervous system and the ductless glands. Martin and others have suggested that it is through these that such a variation in B.M.R. might be expected to take place following a change in environment.

The probability that a variation of endocrine balance would, if responsible, bring about an alteration of the pulse rate or blood pressure in the subjects who exhibit the reaction in B.M.R. can be examined in the experimental results of the present investigation. The conclusion is suggestive but not entirely satisfactory because, although a corresponding change is shown to be present in the circulatory values in the two subjects examined at intervals (Table III), no such constant variation can be shown in the individual results of the subjects examined at two separate periods only. Among the latter also the fall in blood pressure or change of pulse rate is not confined to those who exhibit a definite depression of B.M.R. In view of the fact however that, within normal limits, B.M.R. and circulatory values do not show a corresponding variation from day to day, it might hardly be expected to demonstrate such parallelism in our subjects.

If the tropical environment causes a variation in the nervous or endocrine system it will thereby bring about a modification of the sympathetic-parasympathetic balance, and it has been frequently suggested, though not always accompanied by very convincing evidence, that such disturbances of the sympathetic-parasympathetic balance modify the blood picture. Congo [1927], who studied the effect of drugs upon the leucocyte count, believes that sympathetic preponderance is characterized by an increase in neutrophils and diminished lymphocytes, and parasympathetic preponderance by the reverse. This is also the opinion of Camp [1927] and others. On the other hand, a lymphocytosis is known to occur with stimulation of the sympathetic [Martin, 1932; Menkin, 1928], but this appears to be a more immediate reaction, due to the addition of lymphocytes to the circulation from the spleen and lymph glands. Changes in the polymorph-lymphocyte ratio under altered environmental conditions is claimed to have been found in those living in tropical climates [Chamberlain & Vedder, 1911; Langen & Lichtenstein, 1923; Sundstroem, 1926; Kennedy, 1937]. If those who react to a tropical environment show a change of this nature with parallel variations in B.M.R. and circulatory values, this might form another manifestation of a nervous and endocrine variation.

A comparison of the results obtained for the lymphocyte-polymorph ratios of our group A subjects after different periods of tropical residence (Table II) provides no conclusive evidence on this point. More satisfactory results might have been obtained from counts made upon four successive days, after the system employed in the other measurements, so as to obtain an average basal value, but it was not felt advisable to take blood after a preliminary experimental period in case the true basal values might be influenced and affect the other measurements, which formed the primary object of the investigations.

In the case of the two subjects examined at intervals on the other hand the numbers of polymorphonuclear neutrophils and lymphocytes show a variation which is sufficiently consistent to suggest the possibility of a progressive influence (Table III). In both these cases the subjects were well used to laboratory procedure of the taking of blood and the basal state gave values which might be regarded as more comparable with other samples taken at different times from the same individual under identical conditions. There appears to be sufficient significance in the variation shown in those two cases to justify continuation of further investigation upon this aspect.

Comparison of the average blood pressure with values in temperate climates on the same basis as the B.M.R. is not at present advisable because, although these measurements were also taken with strict observance of basal conditions and could be compared on the basis of weight or surface area with similar measurements made elsewhere, there is no satisfactory standard for temperate climates based upon a sufficiently large number of basal observations, the majority of the standards given being non-basal.

SUMMARY

1. The basal metabolism shows a definite reaction to tropical environment in certain normal individuals and is absent in others.

2. The reaction consists of a gradual fall in the B.M.R. and a corresponding variation in pulse rate and systolic and diastolic blood pressures has also been shown in certain cases.

3. The depression in metabolism in those subjects affected in this way, appears to reach a maximum before the end of the first year in the tropics. This lower value is shown to be maintained after 2 years in the tropics and was not affected by a period of military training in the subjects examined.

4. The environmental factors which may influence these values are discussed. It is concluded that climatic rather than dietetic or occupa-

tional influences are primarily responsible for the variation and evidence is given to support the nature of the physiological change which takes place of which lowering of the B.M.R. is a feature.

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EFFECT OF LIGHT ON RED BLOOD CELLS.
THE LIGHT SENSITIVITY OF BLOOD FROM
DIFFERENT VERTEBRATE SPECIES

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INVESTIGATIONS into the life span of erythrocytes have established the fact that the red cells have an obvious sensitivity to normal daylight [Meyerstein, 1928]. Suspensions of red cells in saline exposed to daylight haemolysed much sooner than similar suspensions stored in the dark. Earle [1928] at the same time reported that electric light haemolysed rabbits' corpuscles.

An extension of this research [Meyerstein, 1928, 1929, 1932] indicated that the haemolytic effect of daylight or artificial light differed quantitatively on blood drawn from different animal species. This observation has now been extended and the technique refined, so that the experiments may be done with one drop of blood.

METHOD

The apparatus used is shown in Fig. 1. The light source is a projection lamp of 500 W, the filament of which is fixed at the focus of the parabolic concave mirror *A*. The light thus collected is reflected to another parabolic mirror *B*, which in turn brings the light to a focus, at which point the small flask containing the blood suspension is fixed. The flask is immersed in a glass vessel cooled by running tap water.

0.05 c.c. blood is taken without anaesthesia from capillaries or veins (it is immaterial which is punctured), and diluted with isotonic saline up to 5 c.c. No anticoagulant is used, the dilution being sufficient to prevent coagulation. 2 c.c. of this 1% blood suspension are placed in a small flask with a long neck, the bulb of which is of 2 c.c. capacity and corresponds in size with the filament of the electric lamp. The bulb has two flat parallel sides. The flask is brought into the focus of the

parabolic mirror *B*, and is exposed to the light for 15 min. During this time the temperature of the blood suspension is taken every 5 min., and has never been found higher than 25–30° C.

After exposure to light, the result is not at once manifest; some time must elapse before the haemolysis produced by the light can be detected. Hence, after exposure to light, the flask is kept for exactly 24 hr. in darkness at room temperature (18–19° C.), and the degree of haemolysis is then estimated. For this purpose, the flask is centrifuged, and the

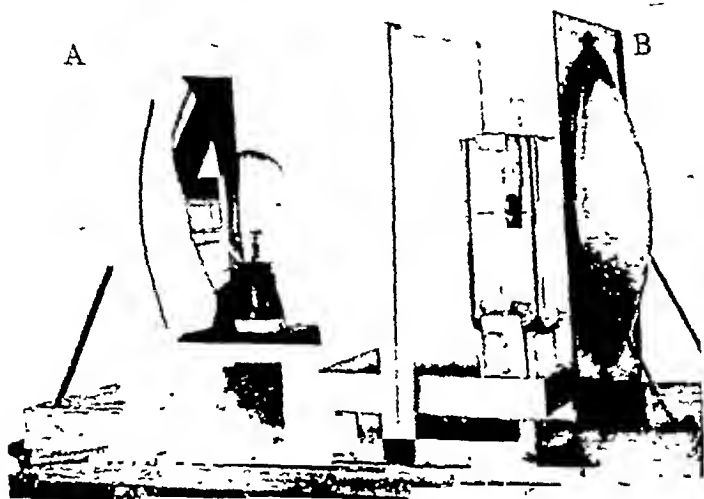


Fig. 1.

supernatant fluid compared colorimetrically with the remainder of the blood suspension, completely haemolysed by freezing or by the addition of a small amount of powdered saponin.

The erythrocytes of the following species have been investigated: man, guinea-pig, rabbit, rat, dog, cat, ox, pig, horse, monkey, hen, frog. All the animals were kept under normal conditions in daylight. In some cases many individuals were investigated; in the others at least two animals of every species were tested, and of every animal two blood samples.

Table I shows the results obtained. Those for the dog and horse were obtained using round bulbs containing 10 c.c. and exposing to light for 1 hr. Experiments on the blood of other animals show that the results obtained by this variation in technique are comparable; therefore it is

considered justifiable to include these results in the table. The results are averages, and with hens the fluctuations are larger than usual, the limits being noted in the table. The blood of the guinea-pig shows a remarkable constancy; therefore this blood was regularly used as control test.

TABLE I

	Degree of haemolysis produced by light under the same conditions %
Frog	5
Hen	15-35
Monkey (<i>Rhesus</i>)	0
Cat	7
Human being	12
Pig	45
Rat (white)	80
Rabbit	80
Ox	85
Horse	90
Dog	95
Guinea-pig	98

The table shows that the nucleated erythrocytes of the hen and frog have a low sensitivity to light. Among the mammals we find extreme differences. There is a large group, guinea-pig, rabbit, rat, dog, ox, and horse, which are highly sensitive to light, with degrees of haemolysis from 80 to 98 %, and another group, man, rat and monkey, which are highly resistant to the haemolytic action of light, with a degree of haemolysis from 0 to 12 %. The strongest resistance is found in the monkey with no haemolysis. But this resistance is not absolute, for if the time of exposure to light is prolonged haemolysis can be obtained. An intermediate position between the two groups is taken by the blood of pigs with a degree of haemolysis of 45 %.

The reasons for the differences in behaviour of erythrocytes of different species when exposed to light are difficult to explain. Plasma plays no obvious part in haemolysis produced by light. The corpuscles were removed from the plasma by repeated washing in isotonic saline, and the light resistance of the washed corpuscles estimated. The following table compares the effect of light on the unwashed cells with that after washing.

TABLE II

	Before washing %	After washing %
Rabbit	80	90
Pig	45	60
Man	15	25

These results indicate either that the diluted plasma gives certain protection, or what is more probable, that the repeated washing of the red cells has produced trauma. Snapper [1912] stated that the osmotic resistance of red cells is diminished by washing; this supports the latter hypothesis. Lepeschkin [1931], also, reported that if erythrocytes were slightly damaged by exposure to hypotonic saline, the haemolysis produced by light increased.

The effect of light on corpuscles of different species bears no relation to their osmotic resistance, as is shown in Table III. The figures for osmotic resistance have been obtained from Isaacs in the *Handbook of Haematology* [1938].

TABLE III

	Osmotic resistance	Haemolysis by light %
Man	0.42-0.48	12
Guinea-pig	0.42	98
Monkey	0.46	0
Dog	0.46	95

Experiments have been done to investigate the nature of the rays which actively produce haemolysis. Ultra-violet light can be excluded (1) because the light is filtered through several layers of glass before impinging on the cells, and (2) because previous experiments [Moyerstein, 1929, 1932] showed that the use of uviol glass instead of ordinary glass in the apparatus, did not affect the results. Infra-red rays are absorbed in part by the cooling water. To test this, however, the flask containing the suspension of guinea-pig blood was immersed in a solution of iodine in carbon bisulphide. By this method almost all rays except infra-red rays are excluded. The haemolysis produced in this experiment was 8% compared with the control test of 95%. Therefore it can be stated that the major effect of light on red cells is produced by rays of the visible spectrum. Further experiments are in progress to determine the factors concerned in this action of light on erythrocytes.

SUMMARY

1. The red blood cells have an obvious light sensitivity, since haemolysis is produced by light. This sensitivity is small with the nucleated erythrocytes of hens and frogs. Among the mammals, two groups are distinguishable, the first with high sensitivity, guinea-pig, rabbit, rat, dog, ox, horse, and the second with big resistance, man, cat, monkey. An intermediate position between these two groups is taken by the blood of pigs.

2. The light haemolysis is caused by alteration of the red cells, not of the plasma.

3. The different behaviour to the action of light has no connection with the osmotic resistance of the erythrocytes.

4. The light which is responsible for the action described is almost exclusively in the visible region.

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The effect of fat on agglutinin titre. By H. C. STEWART.¹ *From the Physiology Department, St Mary's Hospital Medical School, London*

There are two groups of agglutinins, those normally present in human blood or isoagglutinins, and those of bacterial origin. Agglutinin titre may vary in the normal subject, though the cause of this alteration is not known. Milky samples of serum often have an agglutinin titre less than that of serum taken from the same subject only a few hours earlier. Opaque serum is generally associated with a rise in blood fat, as shown and measured by the chylomicrograph technique [Frazer & Stewart, 1939].

The variation in titre of isoagglutinins over a number of hours appears to coincide with the post-ingestive phase of a fat-containing meal and its consequent lipaemia. Consecutive hourly specimens in a fasting subject show a constant resting blood-fat level, and this is accompanied by a similarly constant titre of agglutinin. Each significant rise in blood fat coincides with a fall in agglutinin titre, and the latter rises again as the blood-fat level declines. This effect has been a constant finding with the α and β isoagglutinins, and also with those in the Paul & Bunnell test in cases of glandular fever.

The chylomicron consists of a neutral fat nucleus with an adsorbed globulin film at the oil/water interface [Elkes, Frazer & Stewart, 1939], and as agglutinin is associated with this protein fraction, some alteration in agglutinin titre in lipaemia might be expected. *In vitro* the titre of agglutinin in serum containing little fat can be reduced by one half through the addition of small amounts of a finely dispersed oil-in-water emulsion [Frazer & Walsh, 1933]. For this to occur the fat must be particulate, as a broken or coarse emulsion has little effect, in fact no more than is produced by the addition of the water phase alone. It is suggested, therefore, that the mechanism is one of adsorption of agglutinin at the oil/water interface.

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in the Hb level daily, and clinical jaundice with indirect Van den Bergh reaction appeared. Curve 1 is before treatment, curve 2 when haemolysis was actively proceeding, and curves 3 and 4 when haemolysis had ceased and the Hb level was rising rapidly. It is suggested that these curves provide evidence that microcytosis precedes destruction of the red cells, a macrocytosis occurring when active destruction ceases and intense regeneration is occurring.

The effect of muscular contractions and of curarine on acid gastric secretion in cats. By W. FELDBERG and B. HOLMES.
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According to Anrep and his co-workers [1935, 1939] histamine is released into the plasma from skeletal muscles during their contractions and after curare. The possibility of histamine (or H-substance) acting as a vaso-dilator in the working muscle had already been suggested by Lewis & Grant [1926]. Since a gradual release of histamine over a long period should cause acid gastric secretion, we have carried out estimations of gastric free HCl during muscular work and after curarine.

In spinal cats, previously starved for 15-18 hr., the stomach, according to the method of Edkins [1906], was tied at the cardia and filled with 10 c.c. of saline through a cannula tied into the pylorus. The saline was replaced every 30-40 min. and titrated for free HCl. Under these conditions, provided that the artificial ventilation was sufficient, no HCl was secreted into the stomach.

Histamine. The subcutaneous injection of 50 μ g./kg. usually caused the secretion of free HCl amounting to between 5 and 50 c.c. N/100 HCl. The secretion lasted usually for about 2 hr. and the maximum occurred in the second half hour. Cats under chloralose anaesthesia could not be used since the chloralose inhibited the histamine acid secretion.

Decerebrate rigidity. In decerebrate cats the development of strong and persistent rigidity was associated with the gastric secretion of small quantities of free HCl, amounting to not more than 0.02-0.2 c.c. N/100 HCl per min. It was this observation which initiated the following experiments.

Stimulation of the nerves supplying the legs. The peripheral ends of the cut nerves of both hind legs or of all four limbs were stimulated in rotation with a strong current from an induction coil for 40-70 min. In two experiments the motor roots to the hind limbs were stimulated.

Care was taken to keep the arterial blood well oxygenated. During and after the stimulation the stomach secreted free HCl. In some experiments this amounted only to 5, in others to nearly 200 c.c. *N*/100 HCl. Amounts of over 100 c.c. were obtained when the secretion outlasted the stimulation for a long time. In these instances, the muscles had gone into a state of rigor which, after the end of the stimulation, increased and gradually involved all the leg muscles. As long as this condition persisted the secretion of HCl continued and even increased, diminishing with the disappearance of the rigor or the amputation of the affected limbs.

Curarine. About 20 mg. were injected through a cannula tied into the central stump of the inferior mesenteric artery, the large intestine having been removed. The injection caused gastric secretion of free HCl lasting for several hours, the maximum being reached in the first half hour (about 1–2 c.c. *N*/100 HCl per min.), then decreasing, first quickly, and later slowly.

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PROCEEDINGS OF THE PHYSIOLOGICAL SOCIETY

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The effect of the peripheral resistance on the responses to the intravenous injection of fluid. By R. J. S. McDOWALL

If the arterial and venous pressures of chloralosed cats have been reduced by haemorrhage, the injection of fluid (10 c.c. in 1 min.) results in a rapid recovery of the arterial pressure but the recovery of the venous pressure is very slow.

If, however, the pressures have been markedly reduced by interference with the vaso-constrictor centre or its afferent pathways, e.g. by pithing, the injection of fluid at a standard rate results in a rapid rise in the venous pressure, the arterial recovery being very slow and often negligible. In such animals the venous pressure is, for any given arterial pressure, higher than in an animal which has been bled.

These results are obviously related to differences in the peripheral resistance and may be important in relation to the treatment of shock.

Observations on teat growth in immature female goats. By S. J. FOLLEY and A. C. BOTTOMLEY. (*From the National Institute for Research in Dairying, University of Reading*)

Observations on female kids indicate that for a short time after birth the teats grow isometrically [terminology of Huxley & Teissier, 1936], i.e. at the same rate as the body. This phase is followed by a phase of allometric growth during which the teats grow faster than the body. The earliest observed age of onset of teat allometry was 51 days and the latest 108 days, though in one animal the allometric phase was in progress when observations began at 41 days old. The caprine ovary, therefore, displays endocrine activity quite soon after birth. During the allometric phase the data showed good agreement with Huxley's [1924] simple allometric law.

The goat is a seasonal breeder and exhibits regular oestrous cycles from about September to March. The unexpected observation was made

that in females born in the spring, teat growth ceases completely during the breeding season (in fact in some of our animals slight regression occurred) to be resumed allometrically when the breeding season ends. In the case of two goats born in June the allometric phase extended well into the breeding season, probably because these goats were sexually immature when the breeding season began.

It therefore appears that teat growth is inhibited by some factor associated with the oestrous cycles, probably progesterone secreted by the corpus luteum. Teat and mammary growth are thus distinct processes, at any rate in the goat, since the hormone secretion associated with oestrous cycles promotes growth of the mammary ducts [Turner & Gomez, 1936], but inhibits that of the teat. The effect of oestrogens on the teats resembles the responses of the cervix, sexual skin and vagina of the monkey in that they are all antagonized by progesterone.

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Traumatic shock in experimental cerebral concussion.

By D. DENNY-BROWN and W. RITCHIE RUSSELL

Studies of experimental cerebral concussion without resulting lesions, of which a preliminary note has been made [Denny-Brown & Russell, 1940], have been carried further. It has been found that the phenomenon of concussion is an immediate but transitory paralysis of all bulbar reflex mechanisms examined. The threshold intensity of injury required to induce the effect appears to be identical for a variety of reflexes, though the rate of recovery varies, even in reflexes using the same afferent nerves (e.g. pinna and corneal reflexes). Injury of greater intensity induces more prolonged paralysis, of lesser intensity transient depression of function. Associated with this nervous phenomenon are (1) an immediate jerk or "start" in the musculature, which is identified with the start reflex, (2) bradycardia, a respiratory pause with or without inspiratory spasm, a fall in blood pressure, and motor effects in the limbs and trunk, all traceable to stimulation of the vago-glossopharyngeal system in the medulla, and (3) a transient rise in blood pressure which is related to the phase of traumatic paralysis, and may obscure the fall referred to above.

With sub-concussive injury vagal effects are prominent, and the sudden steep drop in blood pressure for 10–30 sec. closely resembles the effect of the “knock-out” blow. When concussion occurs the period of traumatic paralysis masks the reflex effects from stimulation of the vagus, which then often emerge in the recovery phase. Severe vagal effects may be followed by further slow progressive fall in blood pressure (e.g. from 110 to 60 mm.) in the course of 2 or 3 min. associated with increasingly shallow respiration and loss of spontaneous movement and postural tone, passing into full recovery in a further 2–4 min. This phenomenon can occur in lesser degree in sub-concussive injury, and is considered comparable with “acute surgical shock” from strong stimulation of visceral afferents.

In concussion the immediate vaso-pressor effect may give way to the prolonged shock effect when the injury is severe. Death may then result from a further complete failure of blood pressure occurring suddenly at an interval of 20 sec. to 7 min. after the blow, and associated with very intense peripheral vaso-constriction and rapid pulse. This failure is independent of asphyxia. A sudden concurrent lessening of the volume of the chest indicates identity with the phenomenon of shock under anaesthesia described by Yandell Henderson [1935], and attributed by him to failure of the “veno-pressor system”.

The circulation of the brain is greatly increased in concussion due to stimulation of the depressor nerves as they enter the medulla, but otherwise behaves passively in the above effects.

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Participation of a fat soluble substance in coagulation of the blood. By R. G. MACFARLANE, J. W. TREVAN and ADELA M. P. ATTWOOD. (*From the Wellcome Physiological Research Laboratories*)

It is now well known that acceleration of conversion of prothrombin to thrombin in most samples of horse plasma and many samples of human plasma can be brought about by the addition of various fatty substances. If human or horse plasma, either citrated or oxalated, is either centrifuged at 25,000 revolutions for 2–3 hr., or extracted for 24–48 hr. with carbon tetrachloride, it does not coagulate after the addition

of Russell viper venom and recalcification although it still remains normally coaguable by thrombin. If, however, either lecithin in 10% suspension, the cream from the top of a milk bottle or the fat which rises to the top in the centrifuged plasma is added the coagulation time returns to the normal for the amount of Russell viper venom added. Thrombokinase prepared by extracting lung or brain usually contains enough fatty material to coagulate plasma so treated without addition of fat, although some acceleration is produced by the fat. If, however, thrombokinase is treated with ether, the addition of fat is necessary before coagulation takes place. As Leathes & Mellanby show, pure cephalin is quite inactive. Some of the accelerating substance is found in the deposit from the centrifugation of plasma as well as in the supernatant fatty layer. Partial removal of this factor can be accomplished by filtration through Elford membranes of 0.46 $m\mu$ diameter. (We are indebted to Dr Oakley for the filtration.)

Time of coagulation of 1 c.c. of recalcified plasma

	Alone	Venom	Lecithin	Venom and lecithin	Kinase	Venom and kinase
Horse plasma	540 sec.	30 sec.	330 sec.	12 sec.	120 sec.	—
Horse plasma (extracted)	No clot	No clot	No clot	67	No clot	90 sec.
Human plasma	740 sec.	—	—	—	42 sec.	—
Human plasma (extracted)	>7200	—	>7200	44 sec.	170 sec.	58 sec.

The amount of venom used was 0.01 mg. for each c.c. of plasma. "Kinase" was a watery suspension of dog testis for the horse plasma and of human brain for the human plasma.

Not all samples of lecithin are equally active, showing that the active factor is some impurity. It will be noticed that the fatty material present in the "kinase" suspensions is more effective in restoring the action of Russell viper venom than of the thrombokinase itself.

These experiments provide evidence that, in addition to the known factors in the coagulation of blood, a further factor, fat soluble in nature, is essential.

The effect of cholin-like substances upon epilepsy.

By DENIS WILLIAMS. (Introduced by D. DENNY-BROWN)

A small subcutaneous injection of eserine sulphate given to epileptic patients was found to inhibit *petit mal* epilepsy recorded on the electroencephalogram. Prostigmin and larger doses of eserine were found to

ncrease the number and duration of *petit mal* outbursts [Williams & Russell, 1941]. The effects of other allied substances were, therefore, investigated.

Carbaminoylcholine chloride 0.25 mg. given subcutaneously to epileptics increased the amount of *petit mal* activity occurring spontaneously, or induced by hyperventilation, in seven out of eight experiments. This effect was inhibited by atropine. Acetylcholine hydrochloride (B.D.H.) injected rapidly into an arm vein was followed by an epileptic outburst on four occasions in epileptic subjects. 25 mg. did not cause any change in the electroencephalogram, but 30, 50 and 60 mg. produced electrical changes characteristic of *petit mal* epilepsy in these subjects. In two subjects clinical evidences of the attack were present. The effect was prevented by atropine. Blood-sugar levels, respiratory exchange, and cardiac rate were measured during the experiments, and were found to have no apparent relationship to the *petit mal* outbursts. Control injections in normal subjects were not followed by epileptic activity.

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Vitamin C in cell physiology. By G. BOURNE (*Beit Memorial Research Fellow*). (Introduced by E. G. T. LIDDELL)

Vitamin C can be demonstrated histologically by the use of silver nitrate in 10% acetic acid. The specificity of this reagent has been discussed by various authors [Bourne, 1936; Giroud, 1938].

In many adrenal cortical cells vitamin C is located in the region of the Golgi apparatus [Bourne, 1934, 1935; Leblond, 1934]. It now appears that, in general, vitamin C is present in the Golgi apparatus only of those cortical cells in which [Bennett, 1940] the biologically active 17-keto-steroids are localized. It is claimed [Hirsch, 1939] that whenever a cell is synthesizing quantities of material (e.g. pancreas cell producing zymogen granules) the Golgi apparatus absorbs vitamin C. In the histogenesis of the chick embryo, differentiating cells show a concentration of vitamin C in the Golgi apparatus [Barnett & Bourne, 1941]. Osteoblasts also appear to contain vitamin C in the same region. It has been known for some time that, in scurvy, synthetic processes of cells are inhibited. For example, collagen formation ceases, and therefore wounds heal badly or not at all, and diphtheria antitoxin is not produced [Tonutti, 1939].

The Golgi apparatus is recognized as a synthetic centre of the cell, and the presence in it of vitamin C during active synthesis raises the question as to the part that vitamin C could play in the synthesis of a wide variety of chemical substances. The most likely explanation is that, with increased cell metabolism, the synthetic products would be metabolized as rapidly as they are formed unless they were produced in a specially segregated, highly reducing area of the cytoplasm.

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